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VERGLEICHENDE KARYOLOGISCHE UNTERSUCHUNGEN AN ANTIPODEN

Von

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Einleitung

In den beiden letzten Jahren erschien eine Reihe von Arbeiten, die sich im wesentlichen mit dem Feinbau der Kerne in Antipoden befaßte (HASITSCHKA 1956, HASITSCHKA-JENSCHKE 1957, TSCHERMAK-WOESS 1956 und 1957b, VAZART 1956, FARRON 1957). Es wurden dabei zunächst solche Familien ausgewählt, in denen man — nach Angaben älterer Autoren wie OSTERWALDER (1898), HUSS (1906) und SCHNARF (1902) — hochendopolyploide Kerne in den Antipoden erwarten durfte; so wurden Vertreter der Ranunculaceen und Papaveraceen eingehender untersucht (TSCHERMAK-WOESS 1956 über *Aconitum*, HASITSCHKA über *Papaver*). Die bei ihnen aufgefundenen, sehr auffälligen Ruhekernstrukturen, die in gewissem Maße mit den Riesenchromosomen der Dipteren vergleichbar sind, bildeten den Anstoß zu weiteren Untersuchungen nicht mehr nur an Antipoden (vgl. oben), sondern auch an Synergiden (HÅKANSSON über *Allium nutans*, HASITSCHKA-JENSCHKE 1958 über *Allium pulchellum*, *A. angulosum*, *A. ammophilum*) und an dem Chalazahaustorium von *Rhinanthus* (TSCHERMAK-WOESS 1957a). Allen Veröffentlichungen gemeinsam war das Ziel, Einblick in das Vorkommen und den Feinbau pflanzlicher Riesenchromosomen[1] im speziellen oder ihnen verwandter Gebilde (wie andersartiger Bündel von Tochterchromosomen und Endochromozentren) im allgemeinen zu geben.

Nur in wenigen Fällen nämlich ist *eine einzige* Struktur für den endopolyploiden Kern typisch (regelmäßig nur „Riesenchromosomen" in den Kernen des Endospermhaustoriums von *Rhinanthus*, TSCHERMAK-WOESS 1957a, und „Bündelstrukturen"[2] in den Kernen der

[1] Pflanzliche Riesenchromosomen (oder „Riesenchromosomen") im Unterschied zu den zum Teil etwas andersartigen Riesenchromosomen der Dipteren entstehen im Verlauf der endomitotischen Polyploidisierung, indem die einem Ausgangschromosom entstammenden Tochterchromosomen zu einem Bündel vereinigt bleiben, wobei an ihnen ein Chromomerenbau sichtbar ist.

[2] Als „Bündelstrukturen" werden hier Bündel endomitotischer Tochterchromosomen bezeichnet, die lockerer gebaut sind als die „Riesenchromosomen" und aus etwas dickeren, mehr oder weniger deutlich spiralisierten — und daher keinen Chromomerenbau zeigenden — Chromosomen bestehen.

Synergiden von *Allium ursinum*, HASITSCHKA-JENSCHKE 1957, und *Allium nutans*, HÅKANSSON). Häufiger finden sich neben einem Kernbau, der sich nicht wesentlich von den Strukturen polyploider Kerne anderer Gewebe unterscheidet, noch eine oder sogar mehrere bemerkenswerte Bautypen, die ebenfalls Ruhestadien darstellen (*Papaver* HASITSCHKA; *Aconitum* TSCHERMAK-WOESS 1956; *Allium angulosum*, *A. ammophilum*, *A. pulchellum* HASITSCHKA-JENSCHKE 1958). Diese unterschiedlichen Strukturen hängen einerseits vom Verhalten der Tochterchromosomen nach einer Endomitose — Auseinanderweichen oder Beisammenbleiben —, andererseits vom Grad ihrer Spiralisierung ab.

Die vorliegende Arbeit hat es sich zur Aufgabe gestellt, in erster Linie durch den Vergleich mehrerer Gattungen und Arten aus drei verschiedenen Familien (Ranunculaceen, Papaveraceen, Kompositen) aufzuzeigen, ob und in welchem Ausmaß in endopolyploiden Ruhekernen von Antipoden wechselnde Bautypen hervorgebracht werden, und durch eingehende Untersuchungen dieser Kerne Näheres über den Aufbau und die Verbreitung von Kernen mit „Riesenchromosomen", „Bündelstrukturen" oder Endochromozentren zu erfahren. Weiters konnte die für Antipoden bisher nur von GRAFL (1940) an *Caltha* beschriebene Restitutionskernbildung — allerdings in etwas abweichender Form — festgestellt werden.

Methodik

Das Material stammte aus den Gewächshäusern und dem Botanischen Garten der Universität Wien. Um genaue Analysen der Kernstrukturen durchführen zu können, war es notwendig, möglichst dünne Handschnitte — nur solche ließen sich zu diesem Zweck verwenden — anzufertigen; es wurde also jede Samenanlage in Alkohol: Eisessig = 3:1 kalt fixiert und sofort in dieser Fixierungsflüssigkeit mehrmals parallel zur Mikropyle längs geschnitten[1]. Auf dem Objektträger wurden die Schnitte in Karminessigsäure kalt gefärbt. Die Untersuchungen erstreckten sich auf die Zeit von November 1957 bis Juni 1958. Die stichprobenartigen Kernmessungen wurden in der gewohnten Weise durchgeführt (TSCHERMAK-WOESS und HASITSCHKA 1953); die Volumina wurden nach der Formel für das dreiachsige Rotationsellipsoid berechnet, der Faktor $4\pi/3$ weggelassen.

Die Ranunculaceen

Eranthis hiemalis

Mit der Untersuchung der Antipoden wurde Mitte Januar 1958[2] begonnen. Zu diesem Zeitpunkt war im Embryosack bereits die endgültige Anzahl von 11 Kernen erreicht; jede Antipode ist nämlich normalerweise zweikernig. Nur mehr ganz selten fanden sich die letzten

[1] Wurde die Methodik etwas abgewandelt, so findet sich ein Hinweis im speziellen Teil.

[2] Weniger umfassende Untersuchungen des Jahres 1956 mit etwas abweichenden Resultaten werden am Schluß dieses Abschnittes behandelt.

Mitosen, die diese Zweikernigkeit herstellen. Die Pflanzen sind in diesem Entwicklungsstadium noch sehr klein, die Knospen ragen nur etwa 1 cm aus dem Boden. Ziemlich rasch nach Fertigstellung des Embryosackes setzt in den Kernen der Antipoden endomitotische Polyploidisierung ein, wobei im Stadium der Anthese bis zu 64-Ploidie erreicht wird.

Um die im Verlauf der endomitotischen Polyploidisierung auftretenden Unterschiede im Kernbau (vgl. weiter unten) besser zu verstehen, wurde eine Reihe von Messungen des Kernvolumens durchgeführt. Die errechneten Mittelwerte der Volumina für jede Polyploidiestufe (n bis 64 n) ergaben eine geometrische Reihe mit einem Vergrößerungsfaktor von rund 2 (Tabelle 1). Es wurden wesentlich mehr Kerne, nämlich über 200 genau untersucht, nur ein Teil von ihnen (82) gemessen. Bei nicht meßbaren Kernen ließ sich jedoch der Polyploidiegrad durch Vergleich mit gemessenen ermitteln.

Tabelle 1. *Eranthis hiemalis*

Mittelwerte (M) der Kernvolumina der Antipoden. Werte in μ^3 angegeben (Faktor $4\pi/3$ weggelassen), N Anzahl der gemessenen Kerne, σ_M Abweichung vom Mittelwert

	n	2 n	4 n	8 n	16 n	32 n	64 n
N	3	5	6	10	21	30	7
M	87	260	443	773	1909	3933	7526
σ_M	6,02	5,89	12,61	13,07	159,1	176,9	190,2

Die diploiden Kerne aus dem Nuzellus und den Integumenten enthalten neben einer körnigen euchromatischen Struktur 35—40 kompakte, unterschiedlich große Chromozentren; vier davon liegen dem Nukleolus an (Abb. 1a). Im Unterschied dazu sind in den haploiden und niedrigpolyploiden (bis etwa 4 n) Kernen der Antipoden nur einige wenige, nicht sehr kompakte Chromozentren, von denen zwei mit dem Nukleolus in Verbindung stehen, und das Euchromatin von deutlich fädiger Beschaffenheit zu sehen (Abb. 1b). In den oktoploiden Kernen (in äußerst seltenen Fällen auch schon in tetraploiden) fallen hingegen Unterschiede in der Struktur auf. Bei etwa 70% der oktoploiden Kerne sind die auf dieser Polyploidiestufe noch deutlicher als vorher erkennbaren Chromosomenfäden zu Strängen vereinigt, es zeigt sich also eine „Bündelstruktur" (Abb. 1e), während bei den übrigen die Verteilung im Kern annähernd homogen erscheint (Abb. 1c). Auch alle Übergänge zwischen angedeuteter Zuordnung und augenscheinlicher Bündelung finden sich auf dieser Polyploidiestufe (Abb. 1d und 2). Es handelt sich dabei um das Zusammenhalten der einem Ausgangschromosom entstammenden

Tochterchromosomen. Im weiteren Verlauf der Entwicklung machen sowohl die Kerne mit „Bündelstruktur" wie „homogene" Kerne Endomitosen durch, das Verhältnis 3:7 bleibt annähernd erhalten; es finden sich also auch unter den 16-, 32- und 64-ploiden Kernen noch solche, in

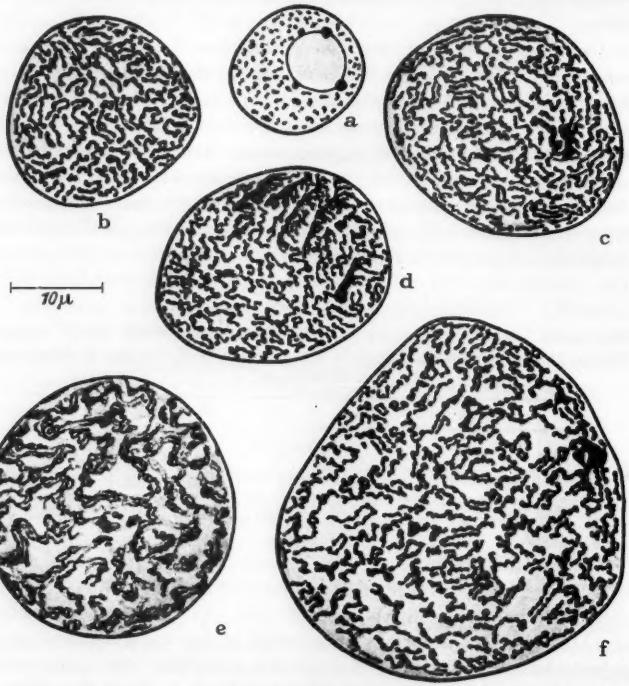


Abb. 1a—f. *Eranthis hiemalis.* a Diploider Ruhekern aus dem Nuzellus; b—f Ruhekerne aus Antipoden; b tetraploider Kern mit gleichmäßiger, ungleich fädiger Struktur; c, d, e oktoploide Ruhekerne; c mit gleichmäßiger, undeutlich fädiger Struktur; d mit Andeutung einer Gruppierung der Fäden; e mit „Bündelstruktur"; f 32-ploider Kern mit nahezu gleichmäßig verteilter, fädiger Struktur

denen die Chromosomen annähernd unabhängig voneinander, höchstens undeutliche Zentren bildend, im Kernraum liegen (Abb. 1f). Als Erklärung dafür, daß in den niedrigpolyploiden (n bis 4 n) Kernen eine Bündelung fehlt, könnte man annehmen, daß die Schwesterchromosomen etwa nur in den heterochromatischen Abschnitten (z. B. in der Region um die Spindelansatzstelle) mehr oder weniger eng zusammenhalten, in den euchromatischen dagegen nicht, so daß der Eindruck einer

homogenen Verteilung der einzelnen Chromosomen im Kern entsteht. In den älteren Kernen könnte sich die Paarung von den heterochromatischen Abschnitten[1] über die gesamte Länge der Chromosomen ausdehnen, diese sich gleichzeitig strecken und dadurch länger werden.

Im folgenden soll nun näher auf den Feinbau der Bündel eingegangen werden; besonders deshalb, weil innerhalb dieses Bautypus mannigfache Abwandlungen vorkommen. Der Anschaulichkeit halber seien zunächst die zwei wesentlichsten Kerntypen herausgegriffen und ihre Struktur näher beschrieben. Es muß aber schon an dieser Stelle betont werden,

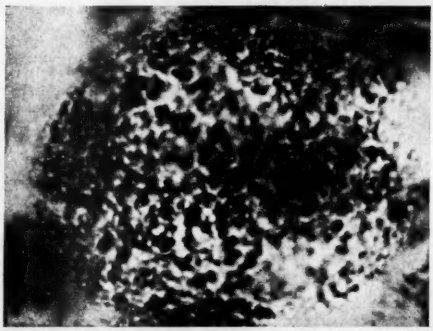


Abb. 2. *Eranthis hiemalis.* 32-ploider Kern aus einer Antipode, die anscheinend teilweise zu Bündeln vereinigten Fäden nahezu gleichmäßig im Kernraum verteilt. Phot. Vergr. 1430fach

daß sich zwischen diesen beiden Ausbildungsformen so ziemlich alle vorstellbaren Übergänge auffinden lassen (Abb. 2). Der in Abb. 3a dargestellte Kern zeigt das charakteristische Aussehen einer „Bündelstruktur" (vgl. Fußnote S. 229). Die Bündel setzen sich aus endomitotisch entstandenen, eng spiralisierten, daher als Fäden sichtbaren Tochterchromosomen zusammen. Sie sind innerhalb eines Bündels locker umeinandergewunden und über ihre ganze Länge lose gepaart. An den Bündelenden wird der Zusammenhalt geringer und an einigen können die Chromosomen sogar spreizen. Stets liegen je 2 Fäden enger beisammen als die übrigen. Die Bündel selbst sind teils gestreckt, teils bilden sie weite, unregelmäßige Schlingen, auch stehen sie bisweilen durch kurze Fadenstücke untereinander in Verbindung. Aus diesem Grund ist die Anzahl der Bündel nicht genau zu ermitteln; höchstwahrscheinlich stimmt sie aber mit der haploiden Chromosomenzahl (n = 8) überein. — Durch Volumenmessungen war es möglich (vgl. oben) den

[1] Über die Verteilung von Eu- und Heterochromatin in den Bündeln vgl. weiter unten.

Polyploidiegrad der Kerne festzustellen. Bei einem Vergleich der Polyploidiestufe mit der Anzahl der Fäden in einem Bündel zeigt es sich, daß niemals die zu erwartende Zahl von Tochterchromosomen zu sehen bzw. zu zählen ist, sondern immer nur halb so viele, d. h. in einem oktoploiden Kern 4 Stränge, in einem 16-ploiden Kern 8 Stränge usw. Man muß also annehmen, daß jeder einfach erscheinende Faden tatsächlich bereits doppelt ist.

An Kernen der geschilderten Bauart (Abb. 3a) fällt weiter auf, daß sich die Unterschiede zwischen Eu- und Heterochromatin weitgehend verwischen. Außer an den beiden dem Nukleolus anliegenden Bündeln (vgl. weiter unten) finden sich keine großen abgegrenzten, heterochromatischen Abschnitte, doch kann man, über die ganze Länge des Bündels verteilt, kleine, intensiv färbbare chromomerenartigen Teilchen und noch kleinere, sich weniger stark anfärbenden Teilchen unterscheiden. Es besteht also ein gewisser Zusammenhang zwischen der Intensität der Spiralisierung und der Ausbildung von Eu- und Heterochromatin. In Kernen mit merkbar spiralisierten Chromosomen, wie sie bei diesem Typus vorliegen (die Chromosomen sind eindeutig als Fäden zu erkennen), fehlt die Ausbildung von deutlichem, kompakten Heterochromatin. Es sei an dieser Stelle auf die von mir (HASITSCHKA) beschriebenen „Riesenchromosomen" bei *Papaver* hingewiesen, in denen — abgesehen von einer wohl vorhandenen submikroskopischen Spiralisierung — die Tochterchromosomen gestreckt sind und die Umgebung des Spindelansatzes deutlich von Heterochromatin markiert ist. — Besonders gut sichtbar werden diese chromomerenartigen Teilchen an mehr *gestreckten* Bündelabschnitten. An einigen wenigen solchen Stellen — mit vorwiegend heterochromatischen Teilchen — wurde eine Struktur beobachtet, die man für Querscheiben nach dem Muster der Dipteren halten konnte: die heterochromatischen Teilchen waren in Querreihen angeordnet, so daß man mit Vorbehalt wohl von einem Scheibenbau sprechen darf (Abb. 3a und c, Pfeile).

Zwei Bündel stehen mit dem Nukleolus in Verbindung (Abb. 3d). Jedes der beiden sitzt mit einem heterochromatischen subterminalen Abschnitt dem Nukleolus auf, wobei noch Fortsätze bis tief in die Vakuolen des Nukleolus reichen. Der dem Nukleolus anliegende Abschnitt kann wechselnd dicht gebaut sein; es können die Tochterchromosomen an dieser Stelle verklebt sein und ein Endochromozentrum oder aber nur eine lockere Gruppe von Fäden bilden (Abb. 3d). Der Vollständigkeit halber sei noch erwähnt, daß in den „homogenen" Kernen zwei mehr oder weniger kompakte Chromozentren dem Nukleolus anliegen.

Eine andere Ausprägung der „echten Bündelstruktur" zeigen auch die Kerne der Abb. 3b und 4a. Ein sehr augenscheinlicher Unterschied gegenüber der bereits geschilderten Bauart liegt allerdings in der Art

Abb. 3a—d. *Eranthis hiemalis.* a, b Ruhekerne aus Antipoden, endomitotische Tochterchromosomen zu Bündeln vereinigt; a 64-ploid, mit zum Teil mehr gestreckten Bündeln (rechts oben — mit Pfeil bezeichnet — Andeutungen eines Scheibenbaues); b 16-ploid, die Bündel zeigen durchgehend deutliche Restspiralen; c Abschnitte dreier Bündel aus einem 16-ploiden Kern, weitgehende Auflockerung des rechten Bündels, Spiralbau im unteren und Querscheibenbau (Pfeil) zeigend; d mit dem Nukleolus in Verbindung stehende Bündelabschnitte aus einem 32-ploiden Kern

der Spiralisierung des Bündels als Ganzes. Waren die Bündel in den eingangs beschriebenen Kern z.T. gestreckt und bildeten nur lockere, unregelmäßige Schlingen verschiedener Länge und Weite, so sind im vorliegenden Fall deutliche Restspiralen vorhanden: die Bündel sind

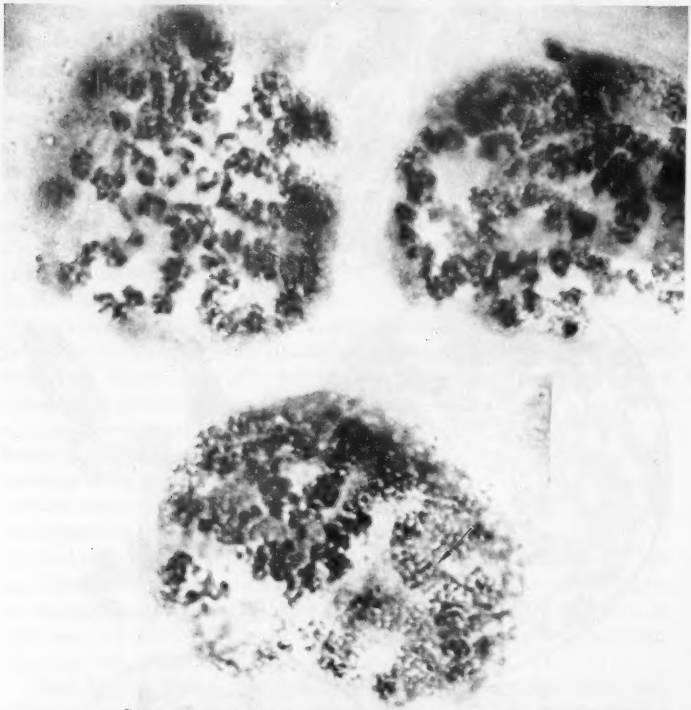


Abb. 4a u. b. *Eranthis hiemalis.* 16-ploide Kerne aus Antipoden mit „Bündelstruktur" (linker Kern der Abb. 4a in der Abb. 3a zeichnerisch dargestellt), bei a Bündel durchgehend, bei b zum Teil in Restspiralen verlaufend, im rechten Teil von b gestreckt. Phot. Vergr. 1430fach

schraubenartig gewunden, wobei Durchmesser und Höhe der Schlingen annähernd gleich bleiben. Gleichzeitig dürften die Tochterchromosomen innerhalb des Bündels enger zusammenhalten als in den mehr gestreckten der zuerst beschriebenen Kerne; auch spreizen sie an den Enden nicht und stehen untereinander vielfach in Verbindung. Die Ausbildung der chromosomenartigen Teilchen tritt zurück und die Tochterchromosomen

haben mehr das Aussehen eines ungleich dicken, unruhig konturierten Fadens (Abb. 3b und 4a).

Daß zwischen den verschiedenen Ausprägungen der „Bündelstruktur" alle Übergänge vorkommen, wurde schon erwähnt. Hinzugefügt sei noch, daß diese Labilität der Struktur so weit führt, daß nicht nur im gleichen Kern, sondern selbst im Verlauf *eines* Bündels deutlich spiralisierte mit fast gestreckten Abschnitten abwechseln und daß sich in seltenen Fällen einzelne Bündeln auflockern, flach ausbreiten und eine Körnchenstruktur deutlich hervortreten lassen (Abb. 3c und 4b). Solche Abschnitte gleichen in ihrem Aufbau manchen typischen pflanzlichen Riesenchromosomen und zeigen eine Folge von größeren heterochromatischen und kleineren euchromatischen Chromomeren, wobei die nebeneinanderliegenden, d. h. also den endomitotischen Schwesterchromosomen angehörenden, heterochromatischen zu scheibenartigen Gebilden zusammenschließen (Pfeile in Abb. 3c und 4b).

Neben den beiden im vorangegangenen geschilderten Strukturtypen findet man in sehr seltenen Fällen noch einen dritten Typus, der allerdings nicht einem Ruhekernzustand entspricht, sondern vielmehr — was sich aus dem Vergleich mit anderen Objekten ergibt (Tschermak-Woess und Hasitschka 1953; Tschermak-Woess 1954; Deufel 1954; Dolež̆al und Tschermak-Woess 1955; Steffen 1955, 1956; Schlichtinger 1956; Hasitschka-Jenschke 1957; Resch 1958) — den Formwechsel der Endomitose darstellt. Die Kerne erhalten in diesem Zustand ein homogen körniges Aussehen, die Unterschiede zwischen Eu- und Heterochromatin sind weitgehend verwischt, bloß eine leichte Zuordnung der chromatischen Elemente bleibt an einzelnen Stellen bestehen (Abb. 5). Da nur etwa 10 Kerne (2%) im endomitotischen Formwechsel aufgefunden wurden, läßt sich nicht entscheiden, ob nicht etwa Ruhekerne mit „Bündelstruktur" und „homogen" gebaute Ruhekerne hinsichtlich der Endomitosestruktur Unterschiede aufweisen. Es wäre denkbar, daß in Kernen in denen die Tochterchromosomen zu Bündeln vereinigt bleiben, die Auflockerung während der Endomitose nicht so weit geht wie in solchen, in denen schon im Ruhezustand ein deutlicher Zusammenhalt fehlt.

Nun sei noch kurz auf die Degenerationsvorgänge eingegangen. Nachdem die höchste Polyploidiestufe (nämlich in den meisten Fällen 64 n) erreicht ist und die Endospermbildung einsetzt, treten langsam Veränderungen der Kernstruktur ein. Die Bündel strecken sich, werden lockerer und verkleben stellenweise. Der ganze Kern macht einen verschmierten Eindruck. In den „homogenen" Kernen wird die Struktur ebenfalls mehr netzig und leicht schmierig.

Weiter wurde versucht, zumindest einen kleinen Einblick in die Faktoren zu gewinnen, von denen die Ausbildung des einen oder anderen

Strukturtypus abhängt. Um Standortsbeeinflussung auszuschalten, wurde nur Material von einer bestimmten Stelle des Botanischen Gartens verwendet. Außerdem wurden Pflanzen getrennt untersucht, die einerseits in einer Periode relativ warmen Wetters (zwischen $+2^0$ und $+6^0$) gewachsen waren, andererseits solche, die starkem Schneefall um 0^0 und weiter solche, die 2 Tage Temperaturen von -6^0 bis -7^0 ausgesetzt waren, schließlich solche, die sich bei frühlingshafter Temperatur (bis

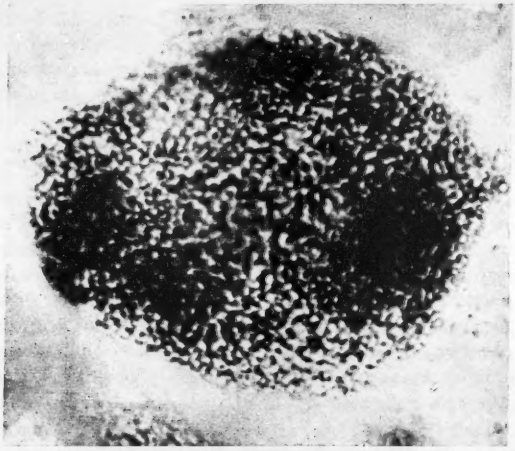


Abb. 5. *Eranthis hiemalis.* In Endomitose befindlicher Kern einer Antipode (von 16- auf 32-ploid). Phot. Vergr. 1430fach

zu $+17^0$) und starker Feuchtigkeit entwickelten. Die Untersuchung brachte (es wurden jeweils mindestens 10—15 Pflanzen durchgesehen) keine Anzeichen für einen Einfluß von Außenfaktoren auf die Ausbildung eines bestimmten Strukturtypus[1]. Kerne mit „Bündelstruktur" und „homogen" gebaute waren in allen Serien in annähernd dem gleichen Verhältnis, nämlich 7:3, vorhanden. Betont sei, daß es sich lediglich um einen Versuch handelte, eine eventuelle *auffallende* Beeinflussung der Ruhekernstruktur durch Außenfaktoren (in diesem Fall nur durch die natürlichen Temperaturschwankungen) festzustellen, und daß sich aus diesem negativen Ergebnis keine endgültigen Schlüsse ziehen lassen. Einen Einfluß von Außenfaktoren anzunehmen, lag einerseits auf Grund von Beobachtungen an *Papaver rhoeas* (HASITSCHKA)

[1] Zur genauen Strukturanalyse, an Hand derer der Kernbau im vorangegangenen beschrieben wurde, verwendete ich ausschließlich Pflanzen der erstgenannten Kategorie.

nahe — wo die Häufigkeit des Vorkommens von 5 Strukturtypen zu verschiedenem Zeitpunkt bei einer Population großen Schwankungen unterliegt, andererseits aber auch auf Grund der Verhältnisse des Jahres 1956 an *Eranthis* selbst; diese sollen im folgenden kurz besprochen werden. Unter den rund 200 damals untersuchten Kernen fand sich keiner, der eine so ausgeprägte Bündelung und Spiralisierung der Tochterchromosomen aufwies wie die Mehrzahl der 1958 untersuchten Kerne. Selten fanden sich Kerne, in denen die nur undeutlich erkennbaren Fäden wohl eine gewisse Zuordnung zeigten aber stark gestreckt waren, so daß offensichtlich mehrere sehr flache „Pseudobündel" oder atypische

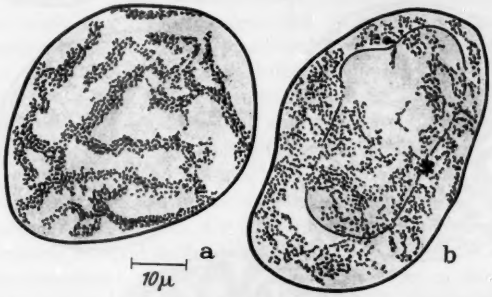


Abb. 6a u. b. *Eranthis hiemalis*. 16-ploide Ruhekerne aus Antipoden (im Januar 1956 untersucht); fädig, körnige chromatische Struktur bei a zu bandförmigen, bei b zu unregelmäßig geformten Arealen vereinigt

„Riesenchromosomen" zustande kamen (Abb. 6a), die untereinander in Verbindung standen. Wesentlich häufiger fand sich ein stark aufgelockerter Typus, bei dem sich fädig-körnige Elemente zu Arealen zusammenfügten (Abb. 6b). Am Nukleolus konnte man zwei dichtere, aus heterochromatischen Teilchen bestehende Areale erkennen. Die diesjährigen Untersuchungen machen es wahrscheinlich, daß es sich bei den geschilderten Strukturen um die Vereinigungsprodukte der stark entspiralisierten, daher undeutlichen, auf dem Wege der endomitotischen Polyploidisierung entstandenen Tochterchromosomen handelt, die zumeist nur einen lockeren Zusammenhalt aufweisen. Auch 1956 konnten Kerne in Endomitosestruktur aufgefunden werden.

Helleborus niger

Die Antipoden von *Helleborus* werden so wie die von *Eranthis* endopolyploid, doch bleiben sie einkernig und erreichen höchstens Oktoploidie, was sich durch stichprobenartige Messungen ermitteln ließ. Meist finden sich in voll entwickeltem Zustand — kurz nach der An-

these — eine tetraploide und zwei oktoploide Antipoden. — Die haploiden Kerne unterscheiden sich in ihrer Struktur kaum von den diploiden aus den Integumenten; sie sind nur größer und etwas lockerer gebaut als

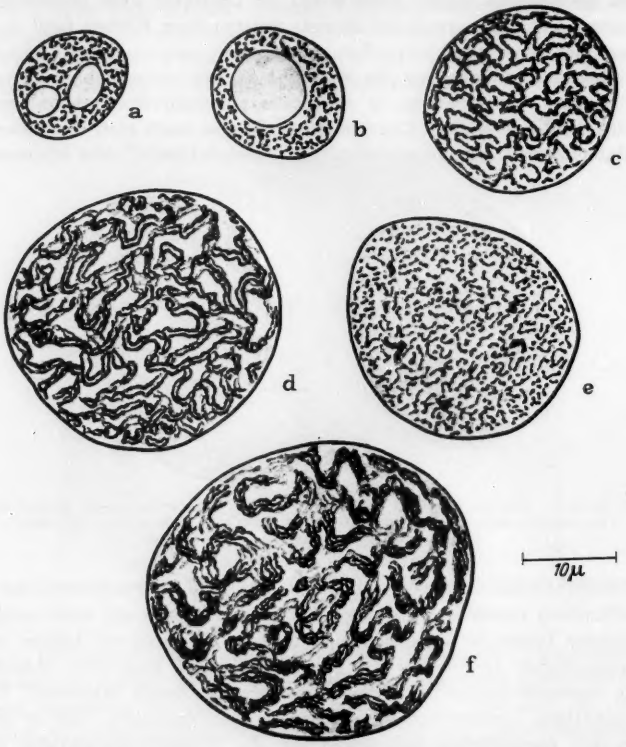


Abb. 7a—f. *Helleborus niger*. a Diploider Kern aus dem inneren Integument; b—f Ruhekerne aus Antipoden; b haploid mit körniger, nur undeutlich fädiger Struktur; c diploid mit deutlich fädiger Struktur; d tetraploid mit zu zweien liegenden, zweiwertigen Fäden; e tetraploid mit gleichmäßiger über den Kernraum verteilter, körniger bis schwach fädiger Struktur; f oktoploider Kern, Fäden gebündelt

diese; sie enthalten körniges bis undeutlich fädiges Euchromatin und einige wenige Chromozentren (vgl. Abb. 7a und b). Wie bei *Eranthis* werden im Zuge der endomitotischen Polyploidisierung zwei verschiedene Ruhekernstrukturen ausgebildet: im häufigeren Fall sind bereits im diploiden Antipodenkern deutliche, d. h. wohl eng spiralisierte, unruhig konturierte Fäden ausgebildet (Abb. 7c); in den tetraploiden Kernen

liegen 2 Fäden ihrer Länge nach parallel oder locker umeinander gewunden; in den oktoploiden Kernen sind 4 Fäden zu einem Bündel vereinigt (Abb. 7d und f). Diese liegen sehr dicht, sind offenbar relativ lang, verlaufen in unregelmäßigen Windungen und in verschiedenen optischen Ebenen, so daß man sie nicht ihrer ganzen Länge nach verfolgen kann. Vermutlich entspricht ihre Zahl in Analogie zum Verhalten anderer Arten der haploiden Chromosomenzahl n = 16. So wie bei *Eranthis* wären in jedem Bündel dann nur halb so viele Fäden zu sehen, als nach dem Polyploidiegrad zu erwarten wären und es müßte auch in diesem Fall angenommen werden, daß jeder einfach erscheinende Faden tatsächlich bereits 2 Chromosomen enthält. Allerdings läßt es sich bei *Helleborus* auch nicht ausschließen, daß 32 Bündel aus einwertigen Fäden vorhanden sind. Ein Unterschied zwischen Eu- und Heterochromatin ist in den Kernen der geschilderten Bauart nicht zu erkennen. In den Kernen jedoch, deren Struktur im Zuge der endomitotischen Polyploidisierung körnig bleibt (vgl. oben) — was viel seltener aufzufinden ist als die geschilderte „Bündelstruktur" — sind wenige, aber deutliche Chromozentren neben einer körnigen, nur undeutlich und kurzfädigen euchromatischen Struktur vom Muster der „somatischen" Kerne zu erkennen (Abb. 7e). Nach den Fällen von *Eranthis* und *Papaver* ist dies ein weiteres Beispiel dafür, daß sich die Unterschiede zwischen Eu- und Heterochromatin mit zunehmender Spiralisierung der Chromosomen mehr und mehr verwischen (auf diese Beobachtung wird später bei der Besprechung der beiden untersuchten Papaveraceen noch zurückzukommen sein).

Anders verhält sich das Chromatin während der Endomitose. Bei diesem Vorgang greift die Entspiralisierung auf das Heterochromatin über, es werden also auch die Chromozentren zerlegt. Allerdings lassen sich in vielen Fällen (vgl. Tschermak-Woess und Hasitschka 1953 und auch das über *Eranthis* Gesagte) noch Areale dichterer Körnchen feststellen, die den heterochromatischen Brocken im Ruhekern entsprechen. — Jedenfalls wird die Kernstruktur während der Endomitose nahezu gleichmäßig *körnig*. Ob die in Endomitose beobachteten Kerne nur von Ruhekernen des zuletzt besprochenen Baues oder von Kernen mit „Bündelstruktur" stammten, läßt sich nicht entscheiden.

Anemone hepatica

Die beiden im vorangegangenen beschriebenen Objekte zeigten den *einen* Weg, der zu echtem Kernwachstum unter Vermehrung des chromatischen Materials in Antipoden führt, ein *anderer* bisher an Antipoden nur von Grafl an *Caltha palustris* genau untersuchter, ist die Restitutionskernbildung. Sie wurde jetzt an *Anemone hepatica* wieder aufgefunden

und dies stellt — wenn auch die Kernvorgänge im einzelnen von denen bei *Caltha* abweichen — eine Bestätigung und Ergänzung dar (Abb. 8a).

Während ältere Autoren (zitiert bei HUSS) Kernfragmentation für verschiedene *Anemone*-Arten angeben, kommt HUSS den tatsächlichen Verhältnissen am nächsten. So soll eine normale Mitose frühzeitig zu zweikernigen Antipoden führen, die im weiteren Verlauf „unreine Karyokinesen" — wie HUSS sich ausdrückt — durchmachen, so daß angeblich bis zu 12 Kleinkerne gebildet werden können. Diese, meint HUSS, verschmelzen allmählich wieder und bilden einen Klumpen in der Mitte der Antipode. Diese Feststellungen und die Angabe DAHLGRENS über den Zeitpunkt der Fertigstellung des Embryosackes unter Ausbildung mehrkerniger Antipoden um Mitte November waren, zusammen mit der neueren Untersuchung an *Caltha palustris* durch GRAFL der Ausgangspunkt für meine Beobachtungen. Die Vorgänge an *Caltha* zeichnen sich durch eine auffallende Regelmäßigkeit aus: nach Fertigstellung des achtkernigen Embryosackes werden die Antipoden zunächst im Gefolge einer normalen Mitose zweikernig, dann kommt es — während einer zweiten Mitosewelle — zur Spindelverschmelzung und es entstehen zwei diploide Kerne je Antipode. Noch zwei weitere Male laufen Mitosen unter Spindelfusion ab und führen am Ende stets zur Entstehung oktoploider Kerne.

Eine solche Gesetzmäßigkeit fehlt bei *Anemone hepatica*. Wohl kommt es regelmäßig — so wie bei *Caltha* — zunächst durch eine normale, haploide Mitose zur Bildung zweier Kerne je Antipode, im weiteren Verlauf der Entwicklung ergeben sich jedoch unterschiedliche Bilder. Ihr Zustandekommen konnte nicht in allen Fällen im einzelnen verfolgt werden, da sich die Teilungsvorgänge (vgl. unten) in den Antipoden über einen langen Zeitraum, nämlich von Mitte November bis Mitte März, erstecken und daher selten aufzufinden sind. Doch lassen sich die folgenden Vorgänge so gut wie sicher rekonstruieren. Die beiden haploiden — und ebenso die älteren, polyploiden — Kerne einer Antipode teilen sich synchron. Je nach dem, ob die beiden Ausgangskerne hintereinander oder nebeneinander liegen, was seinerseits offenbar von der breiteren oder längeren Form der Antipodenzelle abhängt, ist die Stellung der Teilungsspindeln verschieden. In schmalen Antipodenzellen liegen sie schräg hintereinander, so daß zwei nicht zusammengehörige Anaphasegruppen in Kontakt kommen (Abb. 9a und c), außerdem hinken einzelne Tochterchromosomen in der Anaphasebewegung nach, so daß schließlich vier im wesentlichen haploide (vgl. unten) Teilkerne entstehen, die durch schmälere oder breitere Brücken verbunden und serial angeordnet sind (Abb. 10a, unten); auch ein mittlerer größerer Teilkern kann von zwei kleineren flankiert sein und auch völlige Isolierung einzelner Tochterkerne kommt vor. Befinden sich in breiten Antipoden-

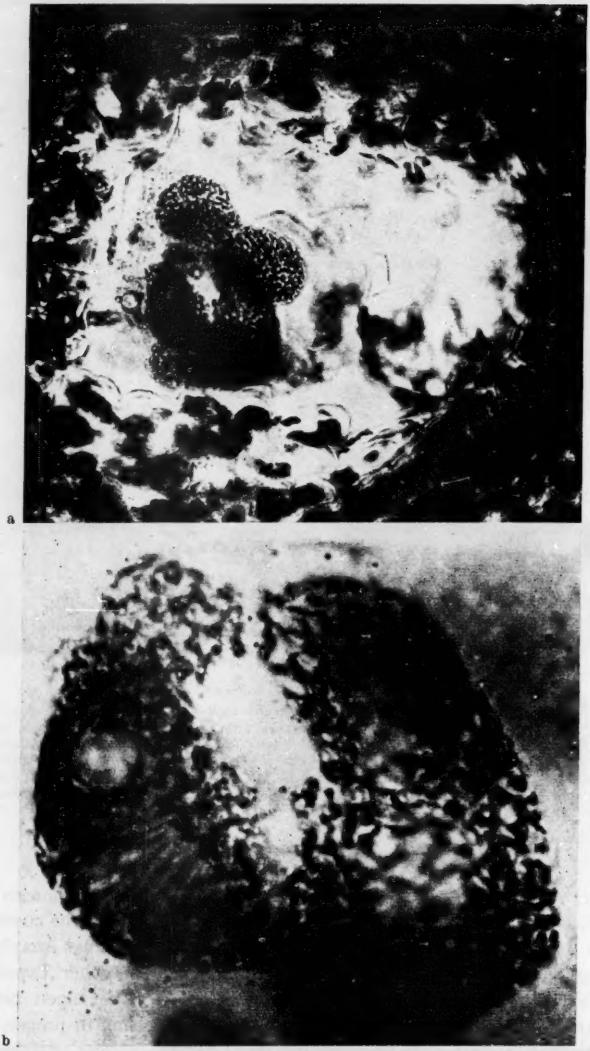


Abb. 8 a u. b. *Anemone hepatica.* a Längsschnitt durch den Embryosack, 3 Antipoden mit Restitutionskernen zeigend; b Restitutionskern aus einer Antipode in früher Prophase (schätzungsweise 16-ploid). Phot. Vergr. a 500fach, b 1430fach

zellen die beiden haploiden Kerne nebeneinander, so verschmelzen offenbar ihre Spindeln seitlich. Dazu kommt gewöhnlich auch noch eine nicht völlig synchronisierte Anaphasebewegung, so daß sich ein tetraploider Restitutionskern bildet, der im wesentlichen aus zwei diploiden Teilkernen besteht (Abb. 10a, oben). Vorgänge nach diesem Muster

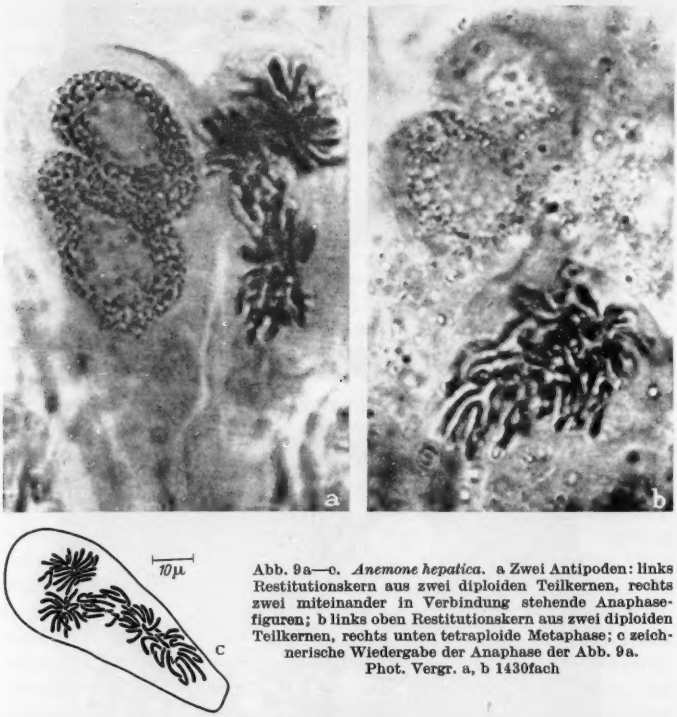


Abb. 9a—c. *Anemone hepatica*. a Zwei Antipoden: links Restitutionskern aus zwei diploiden Teilkernen, rechts zwei miteinander in Verbindung stehende Anaphasefiguren; b links oben Restitutionskern aus zwei diploiden Teilkernen, rechts unten tetraploide Metaphase; c zeichnerische Wiedergabe der Anaphase der Abb. 9a. Phot. Vergr. a, b 1430fach

stellen sich auch bei den folgenden Mitosen ein und es kommen allmählich zum Teil recht kompliziert geformte Restitutionskerne zustande (Abb. 10b). Anscheinend kommt es regelmäßig noch zu einer Anaphasebewegung und setzen nicht etwa nach dem Muster mancher Tapetumtypen (vgl. CARNIEL 1952) die telophasischen Veränderungen bereits im Anschluß an die Pro- oder Metaphase ein. Oft schon in jungen und regelmäßig in älteren Antipoden sind die Teilkerne so eng aneinandergepreßt, daß es sich nicht oder nur schwer entscheiden läßt, ob sie durch eine Brücke verbunden sind; meist wird aber letzteres zutreffen. An-

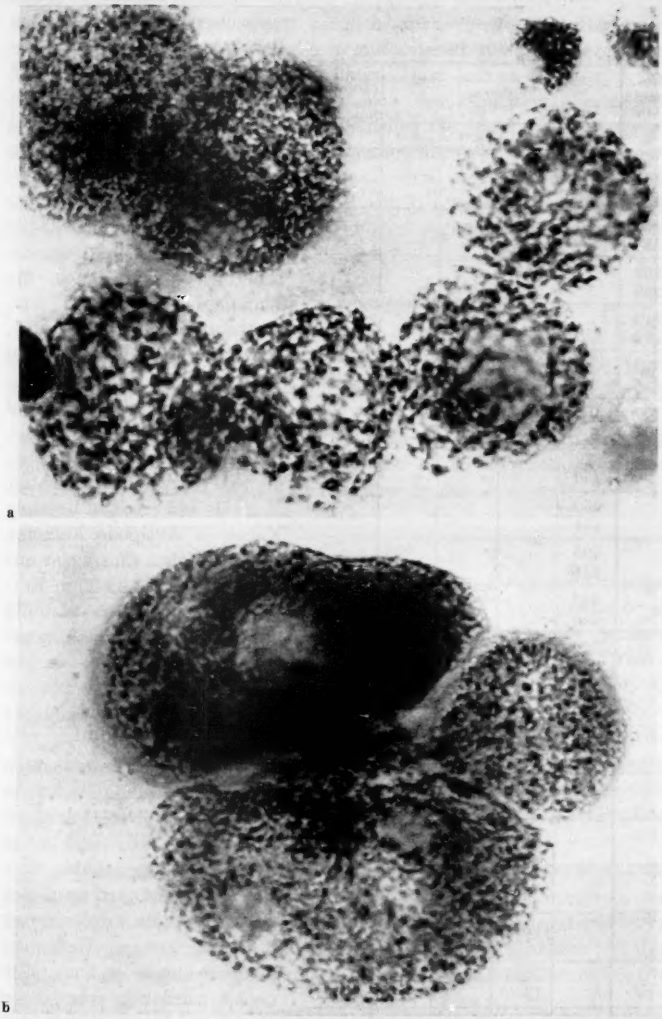


Abb. 10a u. b. *Anemone hepatica.* Restitutionskerne aus Antipoden; a links oben Restitutionskern aus zwei oktoploiden Teilkernen im Ruhezustand, unten Restitutionskern aus vier tetraploiden Teilkernen in früher Prophase; b 32-ploider Restitutionskern aus drei ungleich großen Teilkernen. Vergr. 1430fach

Tabelle 2. *Anemone hepatica*

Werte in μ^3 unter Weglassung des Faktors $4\pi/3$

Getrennte Kerne	Teilkerne				Gesamtpolyploidie
n	2 n	4 n	8 n	16 n	
382 286					n n
300 338					n n
263 260					n n
315 272					n n
229 225					n n
337 225					n n
	572 750				4 n
	650 531				4 n
	495 617				4 n
	640 495				4 n
	722 665	1375			8 n
		1500 1442			8 n
	616 534	1254			8 n
		1470 1330			8 n
			2362 2073		16 n
			2112 2016		16 n
		1188		3264 5304	32 n
		1599 1300	2849		32 n
			2420 1938 2142 2205		32 n

haltspunkte für das Vorkommen einer Verschmelzung von *Ruhe*kernen gibt es nicht. Die Chromosomenverteilung muß trotz der Anaphasestörung im großen und ganzen normal funktionieren, denn in den Anaphaseplatten der polyploiden Teilungsfiguren ließen sich ungefähr ganze Vielfache der haploiden Chromosomenzahl feststellen. Auch die Volumenbestimmungen (vgl. weiter unten) sprechen dafür, daß die Teilkerne einigermaßen vollständige Chromosomensätze enthalten. Maximal sieben am Aufbau eines Restitutionskernes beteiligte Teilkerne wurden beobachtet. Mehr als drei getrennt liegende Kerne je Antipode kommen nicht vor und dies meist nur in frühen und mittleren Entwicklungsstadien, während am Ende der Entwicklung gewöhnlich nur ein aus wenigen oder mehreren Teilkernen zusammengesetzter Großkern vorhanden ist (Abb. 10b).

Von den 180 untersuchten Antipoden befanden sich 24 in Prophase (Abb. 8b), 1 in Metaphase (Abb. 9b) und 7 in später Anaphase (Abb. 9a). Die Beobachtungen an diesen Teilungsstadien wurden durch stichprobenartige Kernvolumenmessungen ergänzt. Zunächst wurden in sehr jungen Embryosäcken die haploiden Kerne solcher Antipoden gemessen, deren Schwester-

zellen sich in haploider Mitose befanden und auch bei älteren Embryosäcken wurde soweit möglich so vorgegangen. Die weiteren Messungen fügten sich in die so gewonnenen gut ein; es ergab sich dabei, daß die Volumina der Teilkerne fast immer annähernd dem Doppelten, Vierfachen oder Achtfachen des haploiden Wertes entsprechen. Wie oben erwähnt, werden also die Chromosomen*sätze* anscheinend einigermaßen regelmäßig aufgeteilt; faßt man die Volumina der Teilkerne einer Antipodenzelle zusammen, so sieht man, daß sie bis zu 32-ploid werden (vgl. Tabelle 2 und Abb. 10b; von den größten Kernen liegen ihrer meist unregelmäßigen Form wegen nur verhältnismäßig wenige Messungen vor).

Die Papaveraceen

Da die Antipoden von *Papaver rhoeas* hoch endopolyploid werden und außer „Riesenchromosomen" andere auffallende Strukturen in ihren Kernen auftreten (Hasitschka), schien die Bearbeitung weiterer Vertreter dieser Familie wünschenswert. Die Wahl fiel zunächst auf *Corydalis cava* und *C. nobilis*, für welche Huss stark vergrößerte Antipoden mit chromatinreichen Kernen angibt[1]. Vollentwickelte Antipoden finden sich bei beiden Arten erst nach der Anthese.

Corydalis cava

Die diploiden Kerne aus dem Nuzellus und den Integumenten zeigen eine körnige euchromatische Struktur und einzelne Chromozentren; ein etwas abweichendes Aussehen haben die niedrigpolyploiden Kerne in den Antipoden (Abb. 11a); in ihnen ist die euchromatische Struktur deutlich fädig, und zwischen 2 oder 4 Fäden besteht eine Lagebeziehung; es finden sich etwa 8 Chromozentren und außerdem sehr kleine Heterochromatinbröckchen im Nukleolus. — Die Erwartung, wie bei *Papaver rhoeas* mehrere Typen von Ruhekernen und vielleicht typische „Riesenchromosomen" aufzufinden, hat sich nicht erfüllt. Die 50 analysierten Kerne zeigten eine im Prinzip übereinstimmende Struktur, die allerdings etwas abgewandelt werden kann.

Im weiteren soll kurz die Struktur höherpolyploider Kerne beschrieben werden. Sie enthalten acht deutliche Chromozentren, was der haploiden Chromosomenzahl entspricht, vier größere und vier etwas kleinere; vier (drei größere und ein kleineres) davon scheinen mit dem Nukleolus in Verbindung zu stehen (Abb. 11b). Die 8 Chromozentren

[1] In bezug auf die Präparation erwiesen sich die beiden Arten als sehr schwierig, die größeren endopolyploiden Kerne platzten nämlich beim leichtesten Druck; lag aber nur eine Zellschicht über ihnen, so drang das Färbemittel sehr schlecht ein. Als relativ günstig erwies sich die Färbung mit Orcein.

sind fast immer auffallend zweiteilig gebaut (vgl. besonders Abb. 12a). Die Aussparung zwischen den beiden Teilen markiert offenbar die Spindelansatzstelle, an der die endomitotischen Tochterchromosomen eng vereinigt sind. Von diesen locker gebauten heterochromatischen Zentren strahlt unregelmäßig sternförmig das Euchromatin in deutlich

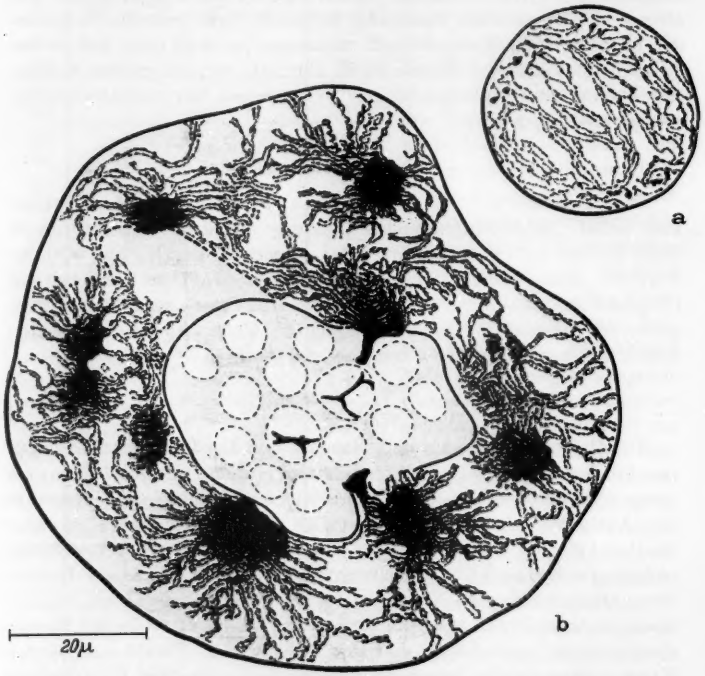
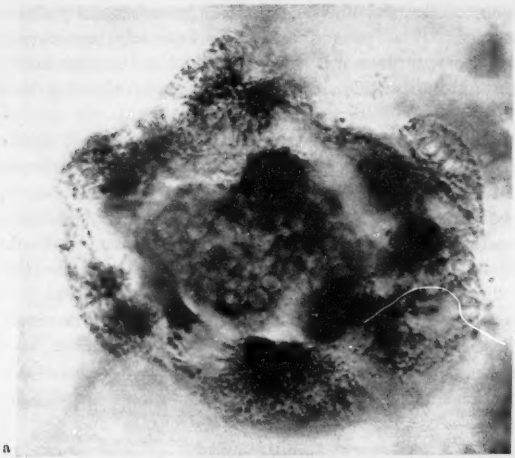
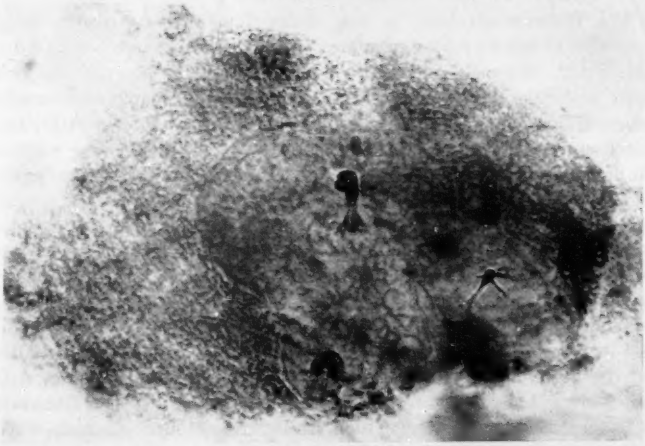


Abb. 11a u. b. *Corydalis cava.* Ruhekerne aus Antipoden; a niedrigpolyploider Kern mit inhomogener fädiger Struktur; b schätzungsweise 64-ploider Kern, von den 8 Endochromozentren strahlten zu zweien liegende euchromatische Fäden radiär aus

fädiger Form aus; daß es sich dabei um endomitotisch entstandene, sehr eng spiralisierte Tochterchromosomen handelt, braucht nach dem schon eingangs Gesagten kaum mehr betont zu werden. Bemerkenswert ist aber wohl, daß die euchromatischen Fäden immer zu zweien beisammen liegen obwohl in diesem Fall offenbar eine gewisse Abstoßungstendenz zwischen den euchromatischen Abschnitten der Tochterchromosomen besteht und sie sich infolgedessen radial ausbreiten (Abb. 11b und 12a); d. h. also, daß ein Zusammenhalt über die *ganze* Chromosomenlänge nur



a



b

Abb. 12a u. b. *Corydalis cava*. Ruhekerne aus Antipoden; a schätzungsweise 64-ploider Kern mit zweiteilig gebauten Endochromozentren, von denen das Euchromatin radiär ausstrahlt; b hochendopolyploider Kern mit Chromozentren aus kompaktem Heterochromatin und engspiralisierten, euchromatischen Fäden. Phot. Vergr. 850fach

zwischen den Tochterchromosomen der *letzten* Endomitose[1] bestehen bleibt und sich die Vereinigung der übrigen, schon früher gebildeten

[1] Oder den *beiden* letzten Endomitosen — falls auch hier jeder Faden 2 Chromosomen enthält — was sich bei dieser Art nicht überprüfen läßt.

Tochterchromosomen auf eine *einzige* Stelle, nämlich die Spindelansatzstelle beschränkt. Das proximale Heterochromatin mit dem die vier erwähnten Chromozentren mit dem Nukleolus verbunden sind, ist tief in ihn eingesenkt, stets finden sich in den einzelnen Vakuolen des Nukleolus auch noch kleinere, heterochromatische Brocken.

Der geschilderte Bautypus kann etwas abgewandelt werden (vgl. Abb. 12b), indem wohl gleichfalls vier kleinere und vier etwas größere Chromozentren vorhanden sind, diese sich aber — im Gegensatz zu den eingangs beschriebenen Kernen — aus *kompaktem* Heterochromatin zusammensetzen. Von ihnen ausstrahlend breitet sich das Euchromatin in undeutlich sichtbaren Fäden weit aus, ist aber — zumindest im Anschluß an die Chromozentren über einen gewissen Abstand — schweifförmig orientiert. An solchen Abschnitten finden sich vereinzelt Stellen mit übereinstimmender Lage chromomerenartiger Teilchen, so daß bisweilen der Eindruck eines Scheibenbaues entsteht. — Auch der für die Endomitose charakteristische Formwechsel wurde an 2 Kernen beobachtet.

Corydalis nobilis

Die Ruhekernstruktur in den endopolyploiden Antipoden weist gegenüber *Corydalis cava* nur wenige, aber doch erwähnenswerte Unterschiede auf. Einerseits zeigt der Vergleich der beiden Arten erneut, wie leicht modifizierbar Ruhekernstrukturen sein können, und andererseits finden einige der an Hand des bisher untersuchten Materials aufgestellten Arbeitshypothesen eine weitere Unterbauung. Der weitaus häufigste Bautypus ist folgender: Es sind acht (n = 8) gegeneinander abgegrenzte Bündel aus endomitotisch entstandenen, offenbar eng spiralisierten und daher dünnen Tochterchromosomen vorhanden; vier stehen in Verbindung mit dem Nukleolus und entsenden Fortsätze in ihn. Deutlich erkennbares, proximal liegendes Heterochromatin markiert die Spindelansatzstelle. Ein nicht mit dem Nukleolus in Verbindung stehendes Bündel ist stets auffallend locker gebaut und enthält wenig und lockeres proximales Heterochromatin (Abb. 13). Da Mitosestadien auf die Zahl ihrer SAT-Chromosomen nicht untersucht wurden, läßt sich nicht entscheiden, ob die vier mit dem Nukleolus in Verbindung stehenden Bündel tatsächlich von SAT-Chromosomen stammen. Wie sich an den hochendopolyploiden Kernen des chalazalen Endospermhaustoriums von *Rhinanthus* zeigt (TSCHERMAK-WOESS 1957a), kann nämlich — anscheinend im Zusammenhang mit einem gesteigerten Umsatz — die Zahl der nukleolenkondensierenden „Riesenchromosomen" größer sein als die Zahl der sonst vorhandenen SAT-Chromosomen (bis zu 17 gegenüber 9 im eigentlichen, triploid bleibenden Endosperm). Jedenfalls besitzen bei *Corydalis* zwei von den vier dem Nukleolus anliegenden Bündeln kompakteres und zwei etwas lockerer gebautes Heterochromatin.

Neben dieser Ausbildung des Ruhekerns findet sich in wenigen Fällen noch eine weitere: Es sind acht wohl als Endochromozentren zu bezeichnende Areale mit radiär ausstrahlenden, euchromatischen, deutlich, wenn auch nicht durchgehend klar spiralisierten Tochterchromosomen vorhanden (s. die durch Pfeile markierten Stellen der Abb. 14). Die vom Nukleolus unabhängigen Endochromozentren sind lockerer gebaut als in den vorher besprochenen Kernen mit „Bündelstruktur".

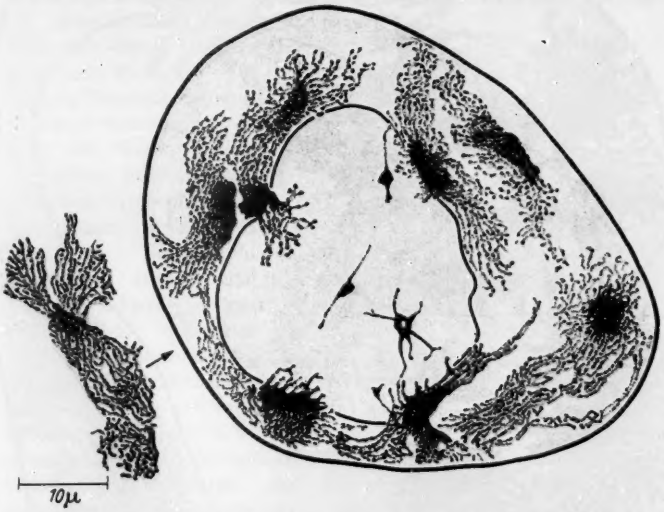


Abb. 13. *Corydalis nobilis*. Endopolyploider Kern aus einer Antipode 8 Bündel aus eng spiralisierten Tochterchromosomen zeigend (ein Bündel der Übersichtlichkeit wegen außerhalb des Kernes dargestellt)

Aus den Beobachtungen ergibt sich erneut ein Hinweis auf den Zusammenhang einerseits zwischen Spiralisierung und Paarung von Tochterchromosomen und andererseits zwischen Spiralisierung und Beschaffenheit des Heterochromatins. In den Ruhekernen mit gut sichtbaren, euchromatischen Fäden, also deutlich spiralisiertem Euchromatin, ist das Heterochromatin lockerer und weniger kompakt ausgebildet als in den Kernen mit wahrscheinlich ganz eng spiralisierten, euchromatischen Chromosomenabschnitten. Auch wurde schon mehrfach die Beobachtung gemacht, daß eine deutliche Spiralisierung der Tochterchromosomen im Ruhekern ihrem engen Zusammenhalt entgegenwirkt; man findet in solchen Fällen eher radiär gebaute Gruppen (*Papaver*, HASITSCHKA) oder locker gebaute Bündel (mehrere *Allium*-Arten, HASITSCHKA-JENSCHKE 1957, 1958). Weiter kann aus den Untersuchungen

an *Corydalis* ersehen werden, daß sich das nukleolusassoziierte Heterochromatin in den Kernen mit radiär gebauten Endochromo-

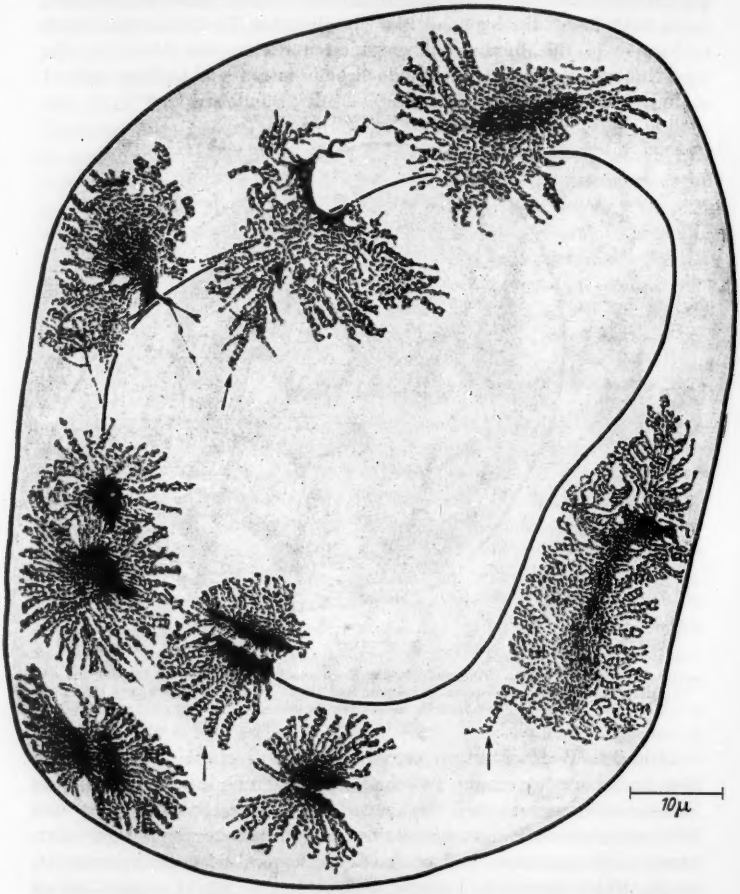


Abb. 14. *Corydalis nobilis.* Endopolyploider Kern aus einer Antipode mit 8 Gruppen radiär ausstrahlender, stellenweise deutlich und relativ weit spiralisierter Tochterchromosomen

zentren weniger stark auflockert als das nicht mit dem Nukleolus in Verbindung stehende, ein Beleg dafür, daß es sich hier um 2 Sorten von Heterochromatin handeln muß. Es sei an dieser Stelle an Endomitose-

stadien erinnert, in denen das Heterochromatin am Nukleolus später und bisweilen auch nicht so vollständig aufgelockert wird wie das im Kern vorhandene übrige (TSCHERMAK-WOESS 1954).

Dicentra spectabilis

Leider degenerierte eine große Anzahl von Samenanlagen schon frühzeitig unmittelbar nach der Ausbildung des achtkernigen, haploiden Embryosackes, und die Fruchtknoten fielen kurz nach dem Verblühen ab (Abfallen der Blüten kurz nach dem Verblühen gibt auch HUSS an). Die übrigen Fruchtknoten entwickelten sich noch weiter, d. h. die Kerne in den Antipoden wurden endopolyploid, zu einem Samenansatz kam es aber in keinem Fall. Diese endopolyploiden Kerne seien kurz geschildert. In dem vorliegenden Material wurde stets nur eine der drei hintereinanderliegenden Antipoden — und zwar die der Mikropyle am nächsten liegende — endopolyploid. Schätzungsweise dürfte nur Okto- bzw. 16-Ploidie erreicht werden. Die 30 von mir untersuchten Kerne zeigten alle einen übereinstimmenden Bau, nämlich acht deutlich abgegrenzte Bündel (n = 8) aus Tochterchromosomen mit proximalem, ziemlich kompaktem Heterochromatin; sie sind den „Riesenchromosomen", wie wir sie von *Papaver* und *Aconitum* her kennen, sehr ähnlich, nur tritt die Fadennatur der Chromosomen gerade noch merkbar hervor. An den euchromatischen Bündelenden wird der Zusammenhalt lockerer und die Chromosomen spreizen (Abb. 15 und 16). Dem Nukleolus

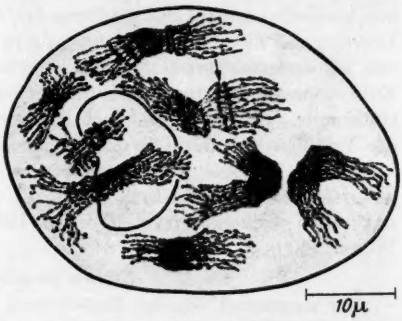


Abb. 15. *Dicentra spectabilis.* Schätzungsweise 16-ploider Kern aus einer Antipode; die endomitotisch entstandenen Tochterchromosomen sind zu 8 Bündeln vereinigt. Einzelne Bündel lassen querscheibenartige Bildungen erkennen (Pfeil)

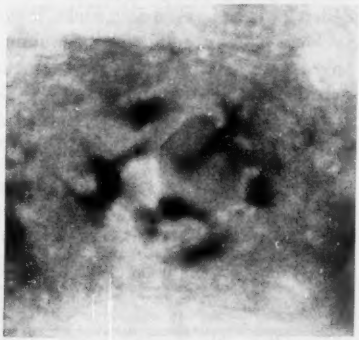


Abb. 16. *Dicentra spectabilis.* Derselbe Kern wie in Abb. 15. Phot. Vergr. 1000fach (Einstellungsebene während der Exposition verändert)

liegen anscheinend stets 3 Bündel an. Ähnlich dem Scheibenbau der Dipteren-Riesenchromosomen lassen sich bei einzelnen Bündeln Stellen auffinden, an denen chromomerenartige Teilchen Querreihen bilden (Abb. 15, Pfeil). Weitere Einzelheiten konnten nicht festgestellt werden.

Kompositen

Vorwiegend ältere Autoren (Literatur bei SCHNARF) haben sich mit der Ausbildung der Antipodenregion bei Kompositen befaßt und die mannigfachen Wege aufgezeigt, die zu der in dieser Familie vorkommenden wechselnden Zahl und Stellung der Antipoden führen. Auf diese Literatur soll hier nur so weit eingegangen werden, als die Angaben die von mir untersuchten Gattungen betreffen oder im Hinblick auf die Entwicklung der Antipoden mit den vorliegenden Untersuchungen übereinstimmen. Hinzugefügt sei noch, daß es mir vorwiegend darauf ankam, das Verhalten der *Kerne* in den Antipoden aufzuklären und etwa vorkommende Endopolyploidie nachzuweisen — was besonders deshalb wesentlich erschien, da Endopolyploidie bei Kompositen anscheinend nur selten vorkommt (vgl. HOLZER 1952, FENZL und TSCHERMAK-WOESS, CZEIKA 1956).

Kleinia ficoides

Der annähernd kugelige Embryosack ist in der chalazalen Region schlauchförmig ausgezogen. Hier kann man zwischen 3 und 8 Zellen, die Antipoden, beobachten; ihre Zellwände können sowohl senkrecht als auch (weniger häufig) parallel zur Längsrichtung der Röhre stehen). Die ursprüngliche Antipodenzahl von drei hintereinanderliegenden Zellen kann also (muß aber nicht, manche Embryosäcke besitzen nämlich auch im Endzustand — wie erwähnt — nur 3 Antipoden) im Anschluß an normale haploide Mitosen vermehrt werden. Nach Abschluß dieser mitotischen Kernteilungen und der Zellvermehrung setzt in allen Antipoden endomitotisches Kernwachstum ein, durch welches bis zu 64-Ploidie erreicht werden kann (Abb. 17a—c). Um den endgültigen Poly-

Tabelle 3. *Kleinia ficoides*

Mittelwerte (M) der Volumina aus den Kernen von Antipoden in μ^3 angegeben ($4\pi/3$ weggelassen); N Zahl der gemessenen Kerne

	n	2 n	4 n	8 n	16 n	32 n	64 n
N	3	1	5	11	5	6	3
M	31	67	109	215	491	845	1711

ploidiegrad feststellen zu können, wurden Kernvolumenmessungen in der üblichen Art (vgl. Methodik) durchgeführt. Sie zeigten — trotz der geringen Anzahl von Messungen — ganz klar ein rhythmisches Wachstum der Kerne, wobei die Mittelwerte jeder Polyploidiestufe einen Vergrößerungsfaktor von annähernd 2 aufwiesen (Tabelle 3).

Eine augenscheinliche Bestätigung für das tatsächliche Vorhandensein von Endopolyploidie ergab sich durch die Auffindung einer spon-

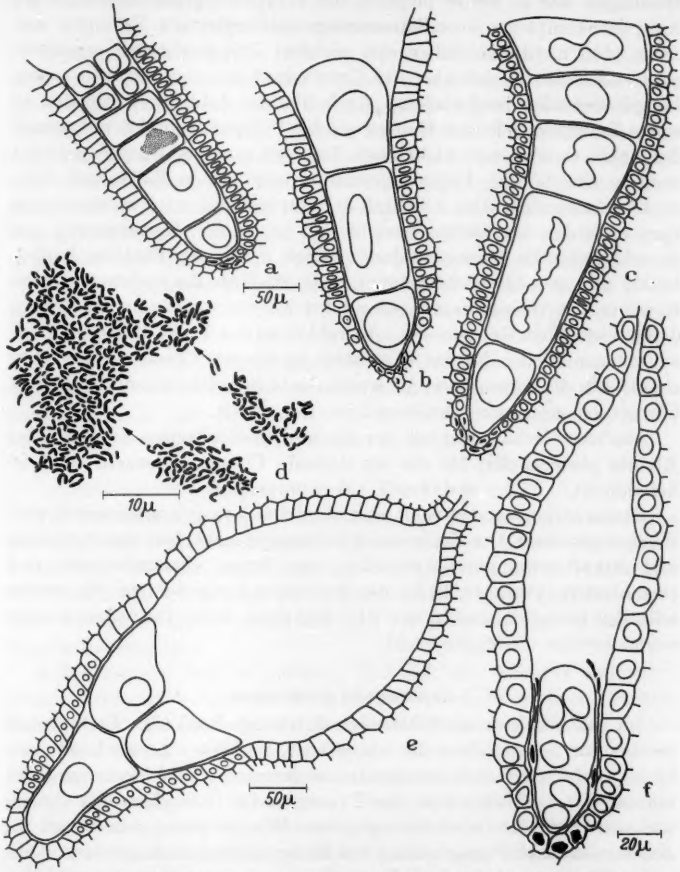


Abb. 17a—f. *Kleinia ficoides*. a—c Längsschnitte durch den Antipodialapparat; in a sind die vier mikropylar liegenden Kerne 4n, die übrigen 8n (punktiert die spontane 8n-Mitose dargestellt); in b sind die beiden mikropylar liegenden Kerne 32n, der chalazal liegende 64n; in c sind alle Kerne schätzungsweise 32n und zeigen die für die Degeneration typischen Veränderungen der Kernform; d spontane oktoploide Mitose (nicht ganz vollständig); e *Othonna crassifolia*. Längsschnitt durch den Embryosack; f *Eupatorium glabratum*. Längsschnitt durch den Embryosack (degenerierende Kerne schwarz dargestellt)

tanen oktoploiden Mitose in einer Antipode (Abb. 17d). So wie in anderen Geweben setzt auch in den Antipoden die Endomitosetätigkeit

gewöhnlich erst nach Abschluß der Mitosen ein, doch wird diese Regel, wie der vorliegende Fall zeigt, gelegentlich durchbrochen. Durch die Messungen war es weiter möglich, die Polyploidiegrade innerhalb des Antipodenkomplexes einer Samenanlage zu vergleichen. Es ergab sich dabei, daß nicht alle Zellen den gleichen Polyploidiegrad erreichen, größtenteils dürfte sich aber der Unterschied auf eine, höchstens zwei Polyploidiestufen beschränken. Auch übt die Zahl der Antipoden in einem Embryosack keinen Einfluß auf den Polyploidiegrad der einzelnen Zelle aus; es wird also nicht etwa dort, wo nur 3 Antipoden gebildet werden, eine höhere Polyploidiestufe erreicht als in Fällen mit zahlreichen Antipoden. Der Abschluß der Entwicklung wird während oder kurz nach dem Aufblühen erreicht und ist an den Kernumrissen gut zu erkennen; die Kerne werden nämlich in diesem Stadium hantelförmig zerzogen bis leicht gelappt. Die Struktur der endopolyploiden Kerne in den Antipoden unterscheidet sich nicht von der in den Kernen der umliegenden Gewebe. Sie ist grobkörnig bis leicht fädig, und nur an wenigen Stellen ist das Chromatin zu kleinen Chromozentren vereinigt. Für die besprochene Art wurde die bisher nicht bekannte diploide Chromosomenzahl von annähernd 100 festgestellt.

Ähnliche Verhältnisse wie bei *Kleinia ficoides* fanden sich auch bei *Kleinia glaucophylla*, für die als diploide Chromosomenzahl rund 90 bekannt ist.

Etwas abweichend verhält sich die dritte von mir untersuchte Art, *Kleinia spinulosa*. Der Embryosack ist langgestreckt, fast zigarrenförmig und enthält meist nur 3 Antipoden: eine lange, chalazaliegende und zwei kleinere, gegen die Höhle des Embryosackes gerichtete; die erstere teilt sich bisweilen noch quer. Alle drei (bzw. vier) Antipoden werden normalerweise niedrigpolyploid.

Eupatorium glabratum

Im achtkernigen, zunächst noch durchwegs haploiden Embryosack werden nur 2 Antipodenzellen abgegrenzt, nämlich eine chalazale einkernige und eine darüberliegende zweikernige. Eine derartige Ausbildung der Antipoden wird von TÄCKHOLM für *Cosmidium* und *Cosmos* und von HOWE für *Grindelia* angegeben. Wie bei diesen setzen auch bei *Eupatorium* nach Fertigstellung des Embryosackes in den beiden Antipodenzellen normale haploide Kernteilungen ein, so daß eine verschieden hohe Zahl von Kernen je Zelle zustande kommt. Während bei *Cosmos* bis zu 40 freie Kerne gebildet werden können, findet man bei *Eupatorium* in der mikropylar gelegenen Antipode nur bis zu 6, in der chalazalen nur bis zu 4 Kerne (Abb. 17f). Ganz selten sind auch drei hintereinanderliegende Antipoden mit je 1—2 Kernen zu finden; in diesen Fällen war es nicht zu entscheiden, ob die Zellbildung schon im Zuge der

Abgrenzung der Antipodenzellen erfolgt war, oder erst nachträglich im Anschluß an eine der zusätzlichen Kernteilungen. Endomitotische Polyploidisierung fehlt.

Othonna crassifolia

Diese Art wurde bereits 1924 von AFZELIUS untersucht. Die 3 Antipoden sind so angeordnet, daß 2 Zellen nebeneinander und die dritte ihnen aufsitzend gemeinsam zu einem keulenförmigen, haustorienartigen Gebilde in die Region der Chalaza auswachsen. Während AFZELIUS angibt, daß die Zellen bisweilen zweikernig werden, die Kerne schließlich aber wieder verschmelzen, fand ich stets nur einkernige Antipoden, deren Kerne auf endomitotischem Wege polyploid werden (Abb. 17e). Nach stichprobenartigen Messungen erreicht der Kern der großen, langgezogenen Zelle Okto- bis 16-Ploidie; die beiden anderen sind immer um 1—2 Stufen niedriger polyploid. Zur Zeit der Anthese ist die Polyploidisierung bereits vollzogen. Die Struktur der endopolyploiden Kerne des umliegenden Gewebes, d. h. das körnig erscheinende Euchromatin, ist mehr oder weniger gleichmäßig über den Kernraum verteilt und außerdem finden sich einzelne Chromozentren. Zu Abwandlungen der Ruhekernstruktur in den Antipoden, wie sie in der vorliegenden Arbeit wiederholt beschrieben wurden, kommt es nicht.

Besprechung der Ergebnisse

Die Untersuchungen der letzten Jahre, die sich die Strukturanalyse endopolyploider Ruhekerne zum Thema stellten, haben eine ganze Reihe von Problemen aufgedeckt, die im folgenden näher zu besprechen sein werden. Es soll dabei nicht versäumt werden, speziell auf die vielen, noch offenen Fragen hinzuweisen und die teilweise noch unsicheren Angaben aufzuzeigen.

Schon längere Zeit ist bekannt, daß sich die Struktur endopolyploider Kerne im Verlauf der Entwicklung innerhalb gewisser Grenzen ändern kann. So beobachteten FENZL und TSCHERMAK-WOESS in der Achse von *Cereus spachianus* in den Kernen zur Zeit des Wachstums Endochromozentren, dagegen in Kernen älterer Abschnitte eine gleichmäßige Verteilung des Chromatins. Ähnliche Angaben macht CZEIKA für das Grundgewebe des Stammes der Sämlinge von *Cereus macrogonus*, für erwachsene Pflanzen und Sämlinge von *Mamillaria centricirrha*, für die Blattepidermis von *Portulaca grandiflora*, für das Stamm- und Blattgewebe von *Anacampseros filamentosa* und für das Grundgewebe des Stammes von *Echidnopsis cereiformis*. Weiter beschreibt SCHLICHTINGER bei *Gibbaeum heathii* für die Kerne der Raphidenzellen Endochromozentren die auf einer bestimmten Polyploidiestufe — ohne Zwischenschaltung spontaner, polyploider Mitosen — in Einzelchromozentren zerlegt werden. Diese Veränderungen sind vom Alter abhängig

und spielen sich im *Laufe der Entwicklung* in ein- und demselben Kern ab. Dagegen finden sich im Blütenbereich, und zwar insbesondere im Embryosack, von *vorneherein* unterschiedliche Bautypen, die wahrscheinlich nicht nachträglich in andere (oder zumindest wesentlich andere) übergehen und die zum Teil außerdem von den Bautypen in vegetativen Teilen auffallend abweichen. — Einen Extremfall stellen die Kerne der Antipoden von *Papaver rhoeas* dar, in denen bis zu fünf unterschiedliche Strukturtypen ausgebildet werden (HASITSCHKA). Daran läßt sich jetzt *Eranthis* reihen mit einer gelegentlich sogar im gleichen Kern unterschiedlichen Beschaffenheit der Chromosomenbündel.

Diese Verschiedenheit in der Strukturausbildung beruht anscheinend im wesentlichen — was schon mehrfach erwähnt wurde — sowohl auf Unterschieden in der Spiralisation der endomitotischen Tochterchromosomen als auch auf solchen der Paarung, wobei zwischen Paarung und Spiralisation auch ein gewisser Zusammenhang zu bestehen scheint: sind die endomitotischen Tochterchromosomen deutlich spiralisiert, handelt es sich also um lichtmikroskopische mehr oder weniger gut auflösbare Windungen, so ist der Zusammenhalt der Tochterchromosomen weniger intensiv als zwischen eng spiralisierten oder weitgehend entspiralisierten. In allen Kernen mit typischen pflanzlichen Riesenchromosomen, wie sie für *Papaver* (HASITSCHKA), *Aconitum* (TSCHERMAK-WOESS 1956), *Rhinanthus* (TSCHERMAK-WOESS 1957a), *Clivia* (TSCHERMAK-WOESS 1957b) und *Allium ammophilum* (HASITSCHKA-JENSCHKE 1958) beschrieben wurden, sind die Tochterchromosomen weitgehend gestreckt und ihrer gesamten Länge nach gepaart. Merkbar spiralisierte Tochterchromosomen sind eher zu lockeren Bündeln vereinigt (*Allium ursinum* HASITSCHKA-JENSCHKE 1957, *Allium odorum* und *nutans* HÅKANSSON, *Eranthis*, *Helleborus* vgl. S. 230—241); und bei bestimmten Kerntypen von *Papaver* und *Corydalis* findet man die zu dicken Spiralen aufgewundenen Tochterchromosomen von einem relativ lockeren heterochromatischen Zentrum radiär ausstrahlen.

Die Hypothese, daß die Menge des Heterochromatins einen Einfluß auf die Ausbildung von „Riesenchromosomen" ausübt oder, im weiteren Sinn, einen Zusammenhalt der Tochterchromosomen fördert (vgl. TSCHERMAK-WOESS 1957b, S. 647), läßt sich, zumindest für die speziell unter diesem Gesichtspunkt untersuchten Synergiden mehrerer *Allium*-Arten nicht halten (HASITSCHKA-JENSCHKE 1958). Allerdings kann man keine ganz allgemein gültigen Regeln aufstellen; das zeigt das Beispiel von *Papaver*: neben den Kernen mit „Riesenchromosomen" und solchen mit Arealen aus spiralisierten Chromosomen, finden sich auch fädige, wahrscheinlich sehr eng spiralisierte Chromosomen, die den Kernraum mehr oder weniger gleichmäßig erfüllen ohne daß es zu einer Bündelung kommt.

Mit der Spiralisierung wechselt auch die Ausbildung des Heterochromatins. Je deutlicher die Tochterchromosomen spiralisiert sind, desto mehr ist der Unterschied zwischen Eu- und Heterochromatin verwischt, was ja auch von den mitotischen Chromosomen her geläufig ist. So sind in den typischen Endochromozentrenkernen, wie sie etwa bei *Sinapis*, *Cucurbita*, *Urtica* vorkommen, die euchromatischen Chromosomenteile im entspiralisierten Zustand vorhanden und nur die heterochromatischen Chromosomenabschnitte sind durch ihre extreme Spiralisierung als kompakte Chromozentren sichtbar. In den Kernen mit „Riesenchromosomen" lassen sich ebenso stets heterochromatische Abschnitte erkennen; auch in diesem Fall sind die Chromosomen weitgehend entspiralisiert. In den Kernen mit „Bündelstruktur" dagegen (mit mehr oder weniger deutlich spiralisierten Chromosomen) sind solche Abschnitte kaum mehr zu finden und sie fehlen schließlich in den Kernen mit noch deutlicherer Spiralisierung.

In diesem Zusammenhang sei auf die Tendenz zur Bildung von Querreihen und Scheiben aus gleichartigen, heterochromatischen Teilchen bei den „Riesenchromosomen" von *Aconitum variegatum*, *Allium ammophilum* und *Clivia miniata* hingewiesen (Tschermak-Woess 1956, 1957b; Hasitschka-Jenschke 1958). Ähnliche Bilder fanden sich zuweilen auch in Kernen von *Eranthis*, *Corydalis* und *Dicentra*, die eine Übergangsform zwischen „Bündelstruktur" und „Riesenchromosomen" zeigten. — In den Kernen der Basiszellen mancher *Helobiae* (Hasitschka-Jenschke unveröffentlicht) finden sich übrigens im Anschluß an die Chromozentren schweifförmige, euchromatische Fortsätze, an denen an einzelnen Stellen ebenfalls Reihen gleichartiger heterochromatischer Teilchen hervortreten.

Bisher kennen wir — wenigstens zum Teil — die *Vorgänge*, die zu den unterschiedlichen Strukturen führen (wie mehr oder weniger deutliche Spiralisierung oder Streckung, Paarung oder Isolierung der endomitotischen Tochterchromosomen) aber nicht die *Faktoren*, die die Ausbildung des einen oder anderen Strukturtypus bewirken. Einiges spricht für die Einwirkung von Außeneinflüssen, anderes dagegen. Während sich 1956 in den Antipoden von *Eranthis* keine klaren „Bündelstrukturen" fanden (vgl. S. 239), wurden 1958 ausschließlich solche gebildet; das Material stammte beide Male vom gleichen Standort. Bei *Papaver* wiederum (vgl. Hasitschka) war das Auftreten der verschiedenen Strukturtypen nach Standorten verschieden: es fanden sich im Material *eines* Standortes stets bevorzugt 1—2 Strukturtypen. 1958 wurde stichprobenartig weiteres Material von *Papaver rhoeas* von verschiedenen Örtlichkeiten Nieder-Österreichs durchgesehen (unveröffentlicht) und der Eindruck gewonnen, daß die Samenanlagen innerhalb *einer* Kapsel vorwiegend 2 Strukturtypen in ihren Antipodenkernen ausbilden, sich

aber unter zahlreichen Kapseln eines Fundortes alle Kerntypen auffinden lassen. Kerne mit „Riesenchromosomen" fanden sich jedoch allgemein sehr selten. Allerdings handelt es sich bei diesen Beobachtungen nur um gelegentliche und nicht streng systematische Untersuchungen.

Die genaue Analyse der Struktur endopolyploider Ruhekerne vieler Objekte hat mehrfach ein *verschiedenartiges* Verhalten des nukleolusassoziierten und des übrigen Heterochromatins gezeigt. Das mit dem Nukleolus in Verbindung stehende Heterochromatin ist meist kompakter und verhält sich anders als das übrige Heterochromatin und auch anders als man nach dem Kerntypus erwarten würde; so bleibt bei *Papaver* in Kernen mit mehr oder weniger gleichmäßig über den Kernraum verteilten, deutlich spiralisierten Chromosomen (Typus IV bei HASITSCHKA), an denen das proximale Heterochromatin nicht mehr zu erkennen ist, am Nukleolus ziemlich kompaktes Heterochromatin erhalten, und auch bei *Corydalis nobilis* haben mit dem Nukleolus in Verbindung stehende Chromosomenbündel dichteres Heterochromatin als die übrigen; weiters wird dieses Heterochromatin oft während der Endomitose weniger weitgehend oder verspätet abgebaut (TSCHERMAK-WOESS an *Sauromatum*). — Auf das Vorhandensein von dichter gebautem und von anschließend etwas lockerem Heterochromatin in einem Chromosom weist TSCHERMAK-WOESS bei der Beschreibung der „Riesenchromosomen" von *Rhinanthus* hin; mit den aus kompaktem Heterochromatin bestehenden Enden stehen mehrere „Riesenchromosomen" mit Nukleolen in Verbindung. In diesem Zusammenhang sei auf die Ergebnisse der Untersuchung von TSCHERMAK-WOESS und HASITSCHKA (1953) hingewiesen, die vier verschiedene Sorten von Heterochromatin in den polyploiden Ruhekernen von Trichomen unterscheiden: 1. kompakt scholliges, mit deutlicher Verklebungs- und Abrundungstendenz (*Cucurbita*, *Ecballium*, *Sinapis*); 2. scholliges, aber anscheinend von weicherer Beschaffenheit und ohne Abrundungstendenz (*Urtica pilulifera*); 3. in Chromomeren gegliedertes (*Urtica caudata*, *Trianea*, *Hydrocharis*); 4. kleine heterochromatische Schollen ohne Verklebungstendenz bei *Sinapis*.

Nachdem GEITLER (1941) für die Wurzelhaarinitialen von *Trianea* und *Hydrocharis* Zustandsänderungen an Zellkernen als Vorgang der Endomitose deutete, konnte durch eine Reihe spezieller Untersuchungen (TSCHERMAK-WOESS und HASITSCHKA 1953; TSCHERMAK-WOESS 1954; DEUFEL, DOLEŽAL und TSCHERMAK-WOESS; STEFFEN 1955, 1956; RESCH u. a. m.) diese Vermutung bestätigt und die Einzelheiten des Ablaufes der Endomitose an pflanzlichen Objekten aufgedeckt werden. Hinsichtlich des Endomitosevorganges an Kernen mit „Riesenchromosomen" und „Bündelstrukturen" läßt sich noch nichts Endgültiges aussagen. Bisher wurde in den Kernen der Antipoden von *Eranthis*,

Helleborus und *Corydalis cava* (vgl. S. 237, 241 und 250) der Synergiden von *Allium ammophilum* (HASITSCHKA-JENSCHKE 1958) und vielleicht auch der Antipoden von *Clivia miniata* (TSCHERMAK-WOESS 1957b) ein Kernbau aufgefunden, der als Ausdruck des endomitotischen Strukturwechsels angesehen werden muß. Sein Erscheinungsbild gleicht dem aus endopolyploiden Kernen anderer Gewebe. Da sich aber einesteils in verschiedenen Geweben und so auch in Antipoden und Synergiden nur 1—2 % der Kerne in Endomitose befinden und andererseits die oben genannten Objekte nur in einem Teil der Embryosäcke „Riesenchromosomen" bzw. „Bündelstrukturen" ausbilden, ließ sich bisher nicht entscheiden, ob sich die Endomitose in Kernen mit „Riesenchromosomen" bzw. mit Bündelstruktur genauso abspielt wie in Kernen mit Endochromozentren und anderen Strukturen. Es wäre ja denkbar, daß Kerne, deren Tochterchromosomen von vorneherein mehr oder weniger frei oder nur locker gepaart liegen, während der Endomitose eine stärkere Auflockerung durchmachen als solche, in denen enggepaarte Chromosomen vorliegen. Beobachtungen hierzu liegen bisher noch nicht vor.

Die Beobachtungen FARRONS an den Kernen in den Antipoden von *Ouratea affinis* sind ein weiteres Beispiel für das Vorkommen von Kernen mit gebündelten Tochterchromosomen. Bei der Strukturbeschreibung gibt der Autor für die Kernperipherie eine mehr prophaseähnliche Struktur, für die den Kernraum ausfüllende, eine mehr ruhekernähnliche an. Nach den Abbildungen und der kurzen Beschreibung zu urteilen dürfte es sich um „Bündelstrukturen" in dem hier verwendeten Sinn handeln, und zwar um Bündel, die der Peripherie genähert liegen und deren euchromatische Enden nach innen ragen, wie es häufig vorkommt, spreizen und sich daher nicht gut verfolgen lassen. Einzelheiten sind der Untersuchung nicht zu entnehmen und so wurde diese und — aus demselben Grund — die noch zu erwähnende von VAZART über den Gametophyten von *Scilla autumnalis* auf dem eine *Ustilago*-Art parasitiert, bisher in der Diskussion nicht berücksichtigt. — In den Antipoden von *Scilla* treten nämlich Kerne auf, die angeblich „etwas mehr als" 12 Bündel enthalten; außerdem finden sich auch Kerne, die denen anderer Gewebe strukturmäßig sehr ähnlich sind. VAZART vertritt nun die — mir recht unwahrscheinlich vorkommende — Meinung, daß die Geschwindigkeit, mit der die endomitotische Chromosomenverdoppelung erfolgt, ausschlaggebend dafür ist, ob die Chromosomen über den Kernraum verstreut oder vereinigt sind. Erfolgt die Verdoppelung sehr rasch, so haben die Chromosomen noch Zeit sich zu ordnen, erfolgt die Vermehrung des chromatischen Materials dagegen langsam, so sind die Chromosomen zu dichten „Paketen" — wie der Autor diese Gebilde bezeichnet — zusammengefügt.

Es sei noch kurz auf die im Bereich des weiblichen Gametophyten vorkommende Restitutionskernbildung eingegangen. GRAFL konnte erstmals mit Sicherheit eine solche für die Antipoden von *Caltha palustris* feststellen und die Vorgänge in den Kernen (zweimalige Mitose mit anschließender Spindelverschmelzung, die regelmäßig zur Ausbildung zweier oktoploider Kerne je Zelle führt) klären. Zu Brückenbildungen und Verklebungen in der Anaphase sowie zur Vereinigung von Telophasegruppen verschiedener Herkunft und zu Spindelverschmelzungen kommt es auch in den Antipoden von *Anemone hepatica* und *A. pulsatilla*, (vgl. S. 242). Innerhalb einer Zelle können solche Vorgänge mehrmals stattfinden; eine strenge Gesetzmäßigkeit wie bei *Caltha* fehlt allerdings. Sehr wahrscheinlich sind alle Angaben über vermeintliche Amitosen in Antipoden und im Endosperm (vgl. MAHESHWARI und die dort angegebene Literatur) als falsch gedeutete Restitutionskernbildung aufzufassen. — Die Autoren (vgl. SCHNARF S. 168) schreiben dem Antipodialapparat eine ernährungsphysiologische Rolle zu; die gleiche Aufgabe hat auch das Endosperm und — im Bereich der Anthere — das Tapetum. Es ist daher nicht weiter verwunderlich, daß auch in diesem Zellverband Polyploidisierung sowohl durch Endomitosen (im zellulär einkernigen Tapetum) als auch durch Restitutionskernbildung (im zellulär mehrkernigen Tapetum) erreicht wird (vgl. hierzu die umfassenden Untersuchungen von CARNIEL). Es ergeben sich also im Bereich der Blüte bei so spezialisierten Zellkomplexen wie den genannten vielfach Parallelen im Hinblick auf die Art und Weise, durch die Polyploidie erreicht wird.

Schließlich sei noch kurz die Frage erörtert, ob die Ausbildung einerseits von Restitutionskernen und andererseits von endopolyploiden Kernen in den Antipoden innerhalb der Ranunculaceen eine systematische Gebundenheit zeigt. Die Embryologie gibt ja ganz allgemein sicherlich Hinweise für die Systematik. In bezug auf das Tapetum haben in letzter Zeit CARNIEL (1952, S. 358) und WUNDERLICH (1954, S. 15) diese Feststellung gemacht; auch MAHESHWARI (1950, S. 358) nennt 12 Kriterien (unter anderem Ausdauer oder Vergänglichkeit von Antipoden und Synergiden), die er für Hilfsmittel in systematischer Hinsicht hält. Außerdem besteht auch zwischen dem Vorkommen von Endopolyploidie und Systematik offenbar eine Bindung, was unter anderen HOLZER, FENZL und TSCHERMAK-WOESS und CZEIKA festgestellt haben. Die grundlegende Arbeit über Antipoden von HUSS, der 18 verschiedene Gattungen der Ranunculaceen untersuchte, die Strukturanalysen von TSCHERMAK-WOESS (1956) an den Antipodenkernen von *Aconitum* und *Delphinium* und die eigenen Untersuchungen (vgl. S. 230—247), bieten einen guten Überblick über die Kernverhältnisse in den Antipoden dieser Familie. Die Angaben von HUSS müssen allerdings auf Grund der jetzigen Erkenntnisse interpretiert werden, doch bietet es keine besonderen

Schwierigkeiten mit Hilfe seiner zahlreichen Abbildungen und seiner ausführlichen Beschreibung zusammen mit eigenen stichprobenartigen Untersuchungen zwischen Endopolyploidie und Restitutionskernbildung zu scheiden; die Gattungen *Paeonia*, *Trollius*, *Helleborus*, *Eranthis*, *Nigella*, *Isopyrum*, *Actaea*, *Aquilegia*, *Delphinium*, *Aconitum*, *Myosurus*, *Ranunculus* und *Thalictrum* bilden offensichtlich normalerweise mehr oder weniger hochendopolyploide Kerne, die Gattungen *Caltha*, *Anemone*, *Clematis* (eigene stichprobenartige Untersuchungen) und — mit Vorbehalt — *Adonis*, zeigen Restitutionskernbildung. *Trautvetteria* mit zahlreichen Antipoden dürfte haploid bleiben. — Zu dem System im Syllabus von ENGLER-DIELS und der neueren Gliederung der Ranunculaceen durch JANCHEN ergeben sich keine augenscheinlichen Beziehungen. Während nämlich *Caltha* unter anderem mit *Helleborus*, *Eranthis*, *Aconitum* und *Delphinium* in beiden Bearbeitungen der Unterfamilie der *Helleboreae* zugezählt wird, bildet *Anemone* mit *Adonis*, *Clematis* und *Ranunculus* u. a. die Unterfamilie der *Anemoneae*. — LANGLET, der der Zahl, besonders aber der Form und Größe der Chromosomen große systematische Bedeutung beimißt, gliedert ebenfalls die vier restitutionskernbildenden Gattungen zusammen mit solchen, die endopolyploide Antipoden besitzen, in drei verschiedene Tribus ein, nämlich *Anemoneae* (z. B. *Knowltonia* mit *Anemone* und *Clematis*), *Trollieae* (z. B. *Delphinium*, *Aconitum*, *Trollius* mit *Caltha*) und *Adonieae* (z. B. *Callianthemum* mit *Adonis*). Für *Knowltonia* und *Callianthemum* liegen allerdings bisher keine Untersuchungen an Antipodenkernen vor. — Es ergeben sich also zwischen diesen beiden grundlegend verschiedenen Wegen der Polyploidisierung (Restitutionskernbildung und Endopolyploidie) in den Kernen der Antipoden und der systematischen Gliederung der Ranunculaceen, wie sie von verschiedenen Autoren durchgeführt wird, keine Beziehungen. Wie bereits mehrfach betont, unterscheiden sich die endopolyploiden Kerne im Embryosack in ihrem Bau häufig sehr auffallend von den endopolyploiden Kernen anderer Gewebe.

Ein weiteres Beispiel dafür, daß sich die Elemente des Embryosackes — zwar nicht in der Struktur, aber im Vorkommen von Endopolyploidie überhaupt — anders als die Kerne und Zellen der übrigen Gewebe derselben Pflanze verhalten können, bieten die hier behandelten Vertreter der Kompositen. Während nämlich Endopolyploidie in dieser Familie anscheinend nur selten auftritt (HOLZER, FENZL und TSCHERMAK-WOESS, CZEIKA), fanden sich in den Antipoden von *Kleinia ficoides* bis zu 64-ploide Kerne (die Antipoden von *Kleinia spinulosa* und *Kleinia glaucophylla* werden etwas niedriger polyploid) und bei *Othonna crassifolia* bis zu 16-ploide Kerne. Nach CZEIKA wird nur das Mesophyll der fleischigen, wasserreichen Blätter von *Othonna crassifolia* bis zu 32-ploid, die Mehrzahl der Mesophyllzellen ist 16-ploid, während die Blätter und die Achse der übrigen Arten diploid bleiben.

Zusammenfassung

Es wurden je 3 Vertreter der Ranunculaceen, Papaveraceen und Kompositen in Hinblick auf ihren Antipodialapparat genau untersucht, wobei der Kernstruktur spezielles Augenmerk galt.

Die Kerne in den Antipoden von *Eranthis hiemalis*, *Helleborus niger*, *Corydalis cava*, *Corydalis nobilis*, *Dicentra spectabilis*, *Kleinia ficoides* und *Othonna crassifolia* machen — nach Fertigstellung des haploiden Embroysackes — eine Periode des endomitotischen Wachstums durch. Die Antipoden von *Anemone hepatica* werden infolge von Restitutionskernbildung polyploid, bei *Eupatorium glabratum* bleiben die an Zahl vermehrten Kerne haploid. Durch — zum Teil nur stichprobenartige — Kernvolumenbestimmungen läßt sich für *Eranthis* 64-Ploidie, für *Helleborus* Oktoploidie, für *Kleinia* 64-Ploidie, für *Othonna* 16-Ploidie und für *Anemone* 32-Ploidie der Kerne in den Antipoden feststellen, wobei eine Antipodenzelle bei *Eranthis* stets zwei, bei den übrigen Arten nur einen endopolyploiden Kern enthält; bei *Anemone* ist die aus dem Volumen der Teilkerne errechnete Gesamtpolyploidie angegeben.

Neben einer mit dem Bau endopolyploider Kerne aus anderen Geweben übereinstimmenden annähernd homogenen, chromatischen Struktur findet sich in der Mehrzahl der endopolyploiden Kerne von *Eranthis* und *Helleborus* noch eine weitere, die ebenfalls einen Ruhekernzustand verkörpert: die Chromosomen sind mehr minder spiralisiert und zu lockeren Bündeln vereinigt; diese können bei *Eranthis* deutliche Reliktspiralen bilden oder aber auch mehr gestreckt sein und lassen dann an einzelnen solchen Stellen einen Querscheibenbau nach dem Muster der Riesenchromosomen der Dipteren erkennen.

Bei *Corydalis cava* finden sich — entsprechend der haploiden Chromosomenzahl — acht, meist lockere, heterochromatische Endochromozentren, von denen das Euchromatin in deutlich fädiger Form ausstrahlt, wobei stets 2 Fäden eine Lagebeziehung aufweisen. — Bei *Corydalis nobilis* sind im weitaus häufigsten Fall acht (n = 8) abgegrenzte Bündel aus endomitotisch entstandenen Tochterchromosomen vorhanden. Selten findet sich ein anderer Bautypus: von einem Zentrum aus sehr locker gebautem Heterochromatin strahlen merklich spiralisierte Tochterchromosomen radiär aus.

Bei *Dicentra* sind in den endopolyploiden Kernen ausschließlich acht (n = 8) deutlich abgegrenzte, „riesenchromosomen"-ähnliche Bündel aus Tochterchromosomen mit proximalem Heterochromatin aufzufinden.

An Hand der Strukturanalyse an Kernen von verschiedenem Typus wird auf die Zusammenhänge zwischen Spiralisierung und Hervortreten des Heterochromatins verwiesen: je deutlicher die Chromosomen spiralisiert sind, desto weniger eng ist der Zusammenhalt der endomitotisch entstandenen Tochterchromosomen. In Kernen mit deutlich spirali-

sierten Chromosomen tritt das Heterochromatin weniger hervor als in solchen mit undeutlich (wahrscheinlich eng) spiralisierten Chromosomen.

Der für die Endomitose charakteristische Strukturwechsel konnte in einzelnen Kernen bei *Eranthis*, *Helleborus* und *Corydalis cava* aufgefunden werden.

Regelmäßiges Auftreten von Restitutionskernbildung wird für die Antipoden von *Anemone hepatica* und *A. pulsatilla* festgestellt. Brückenbildungen, Spindelverschmelzungen und Vereinigung zwischen Telophasegruppen *verschiedener* Teilungsfiguren führen anfangs zu einer Reihe serial liegender, untereinander verbundener Teilkerne, sowie zur Bildung zweier größerer Teilkerne oder eines mittleren, größeren, der von kleineren flankiert ist; später kommen häufig unförmige, aus verschieden großen Teilstücken zusammengesetzte Kerne zustande. Volumenbestimmungen an den Teilkernen ergeben, daß das Volumen meist ein Vielfaches des haploiden Wertes beträgt, so daß man annehmen muß, daß die Aufteilung der Chromosomensätze einigermaßen regelmäßig erfolgt.

Bei *Kleinia ficoides* (stichprobenartig wurde auch *Kl. spinulosa* und *glaucophylla* untersucht) und bei *Othonna crassifolia* treten im Laufe des endomitotischen Wachstums in den Ruhekernen *keine* bemerkenswerten Strukturen auf. Bei *Kleinia ficoides* wird meist die ursprüngliche Anzahl von drei hintereinanderliegenden Antipoden bis zu acht vermehrt. Es wurde eine spontane oktoploide Mitose aufgefunden.

Für *Kleinia ficoides* wird als diploide Chromosomenzahl $2n \sim 100$ angegeben.

Bei *Eupatorium glabratum* wird im haploiden Embryosack stets eine chalazale, einkernige und darüber eine zweikernige Antipodenzelle abgegrenzt. Endomitotische Polyploidisierung fehlt, doch wird die chalazale Antipode gewöhnlich zweikernig, die darüberliegende vier(selten acht)kernig.

Zwischen Restitutionskernbildung und Endopolyploidie in den Kernen der Antipoden und der systematischen Gliederung der Ranunculaceen ergeben sich keine systematischen Beziehungen.

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ON THE CYTOTAXONOMY OF PHASMIDS (PHASMATODEA)*

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With 14 Figures in the Text

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All normally bisexual phasmids thus far studied cytologically have in common a characteristic pattern of male meiosis. In contrast to this uniformity, chromosome number varies widely: the known complements range from 21 to 53 (2n♂). No correlation has yet been demonstrated between chromosome number and the taxonomic categories comprising the order[1]. High, medium and low numbers occur in each of the two included families, and in each subfamily of which several species have been studied a similar range is found. Indeed, the highest and lowest numbers now known are found within the same subfamily (FAVRELLE 1934), and congeneric species with 27 and 47 occur (HUGHES-SCHRADER 1947b). Of special interest with respect to the course of evolutionary change in chromosome number are the *Prisopini*, not hitherto investigated cytologically, since they constitute one of the most primitive groups of living phasmids. In the following report cytological data on two prisopine species are considered in relation to this question.

Materials and Methods

The material comprises 3 adult males of 2 closely related species of *Prisopus*, tribe *Prisopini*, subfamily *Pseudophasminae*, family *Phylliidae* (=*Areolatae* REDT.). Two specimen represent *P. berosus* WESTWOOD, whose known range extends through Panama into southern Mexico; the third is *P. ariadne* HEBARD, known only from Costa Rica and Panama. They were collected at night lights on Barro Colorado Island, C. Z., Panama, in May, 1956 and June, 1957. The specimen are deposited in the Academy of Natural Sciences, Philadelphia; grateful acknowledgement is made to Dr. J. A. G. REHN of the Academy for their identification.

The testes were fixed in Sanfelice, sectioned, and stained with Feulgen (Stowell procedure). Cytophotometric measurements of DNA (deoxyribose nucleic acid)-Feulgen in spermatid nuclei were made with the apparatus and methods described by ANSLEY (1954). The figures are camera lucida drawings; the magnification as reproduced is indicated by a scale on each.

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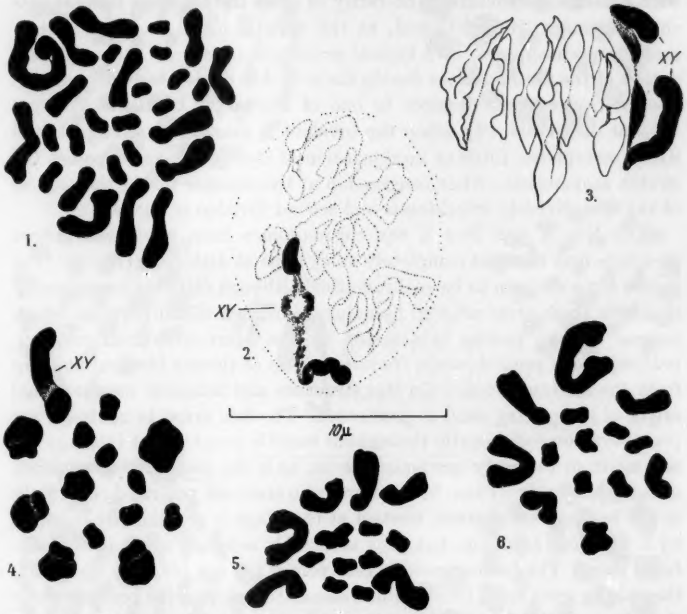
[1] The classification followed is that of GÜNTHER (1953).

Cytological Observations

Prisopus ariadne

The diploid complement of the male numbers 28 (Fig. 1). It includes 2 sex chromosomes whose structure and behavior, as compared with the XO—XX mechanism characteristic of phasmids, identify them as Neo X and Neo Y. One of these is distinguishable at gonial metaphase



Figs. 1—6. *Prisopus ariadne*. 1. Spermatogonial metaphase. 2. Diakinesis, with some bivalents orienting in premetaphase stretch; Neo *XY* shown in detail in lower left. (Not all bivalents drawn in this and following figure.) 3. First meiotic metaphase; Neo *XY* at right. 4. Same, in polar aspect; Neo *XY* at upper left. 5. Second meiotic metaphase; the smaller sex chromosome is one of the 3 large chromosomes with submedian kinetochore. 6. Second meiotic metaphase containing the larger sex chromosome at top center

by its relatively large size and submedian kinetochore (Fig. 1). The second corresponds closely in size and in the subterminal position of the kinetochore with the 2 second-longest pairs of autosomes (Fig. 5). Of the autosomes, 7 pairs are markedly smaller than the rest and tend to take a central position in the metaphase plate; 3 pairs are intermediate in size, and 4 pairs are relatively large. All are mediokinetic, with the exception of the 3 smallest pairs in which no clear evidence of a second arm was demonstrable.

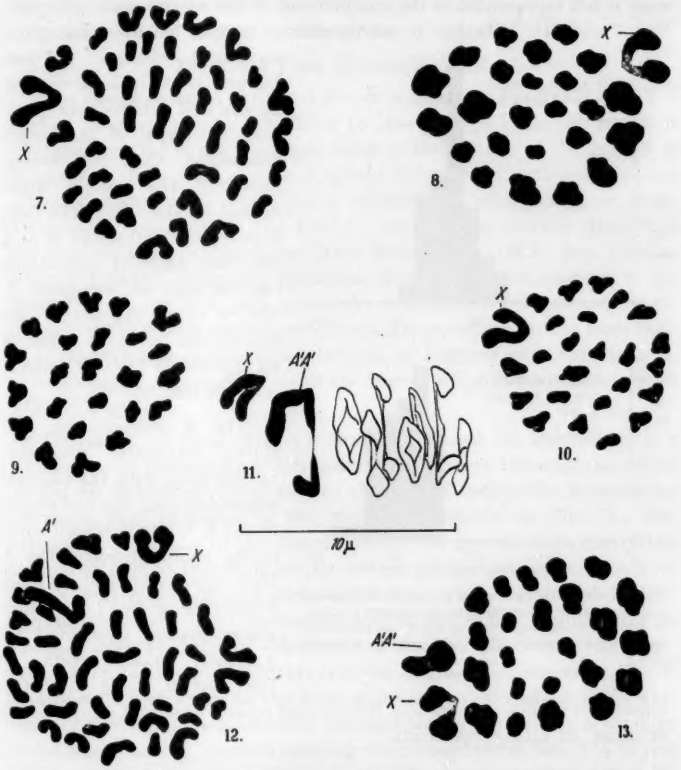
The general course of male meiosis conforms closely to that typical for the group (HUGHES-SCHRADER 1947a), and its major features can be briefly summarized. Early prophase shows a bouquet orientation at leptotene and zygotene which relaxes at pachytene. There follows an extreme extension of the chromosomes to give a "confused" stage of long duration from which the bivalents emerge in diakinetic form and with chiasmate structure. The rarity of cysts in this stage bespeaks its short duration; it is followed, as the spindle forms and the nuclear membrane disappears, by a typical premetaphase stretch stage. Attenuation of the chromosomes during the stretch is moderate. For example, the interkinetochore distance in one of the larger bivalents averages 7.5 μ at diakinesis, 12 μ when the bivalent is maximally stretched, and 6 μ at metaphase. Little or no elongation of the spindle accompanies the stretch movements. After congression at the equator the further course of the first division, interkinesis and second division is regular.

The Neo X and Neo Y sex chromosomes form a bivalent whose structure first becomes completely analyzable at diakinesis (Fig. 2). The paired arms are seen to be euchromatic. Although differing considerably in length these arms are still homologous in their distal portions whose normal meiotic pairing is attested by the open cross configuration, indicative of a prior chiasma, frequently seen as the sex bivalent emerges from the confused stage. On this structure and behavior an autosomal origin of the pairing arms is predicated. The free arms, in contrast, are positively heterochromatic throughout meiotic prophase, at interkinesis, and again in the early spermatid nuclei, as is the single X chromosome of most phasmids. These heterochromatic arms are polarized separately in the bouquet orientation; contact at this stage is occasionally followed by a transient adhesion, but they invariably separate again in the confused stage. The further meiotic behavoir of the sex bivalent is regular; the pairing arms come into terminal association during the premetaphase stretch, and the bivalent orients as do the larger autosomes in the periphery of the equatorial plate (Figs. 3 and 4); Neo X and Neo Y segregate at the first division and divide at the second (Figs. 5 and 6). Until female material is studied, the X cannot, of course, be distinguished from the Y. That the larger chromosome is the Neo X, however, is suggested by comparison with the single X of the closely related species *P. berosus*. The latter is 4.5 to 5 μ in length, which is closely approached by the heterochromatic arm of the larger sex chromosome in *P. ariadne* with an average length of 4 μ, while the free arm of the smaller partner is approximately only 1.5 μ long.

Prisopus berosus

This species, closely resembling *ariadne* in gross morphology, diverges markedly in chromosome complement. *P. berosus* retains the XO—XX

sex chromosome mechanism characteristic of phasmids, and, in contrast to the 28 chromosomes of *ariadne*, the diploid complement of the male numbers 49. It comprises a relatively large mediokinetic X and 24 pairs



Figs. 7—13. *Prisopus berosus*. 7. Spermatogonial metaphase; *X* peripheral at upper left. 8. Polar aspect first meiotic metaphase; *X* at upper right. 9. Second meiotic metaphase, with 24 autosomes. 10. Same, with 24 autosomes + *X*. 11. Lateral aspect of first meiotic metaphase, with *X* and unequal autosomal bivalent in black at left. (Not all bivalents are drawn.) 12. Spermatogonial metaphase from same specimen as Fig. 11; the larger of the unequal autosomes at A^1. 13. Polar aspect of first meiotic metaphase from same specimen as Figs. 11 and 12; *X* and unequal autosomal bivalent (A^1 A^1) at left

of autosomes (Fig. 7). The latter form a series closely graded in size, the largest of which approximates in length the medium sized chromosomes of *ariadne*. Kinetochore position could not be determined in the smaller elements, but at least 16 pairs are mediokinetic.

Structural diversity of certain chromosomes is evident. In one of the two males studied, there is found an unequal pair of autosomes (Fig. 11), whose larger member exceeds even the X in size; this chromosome is not represented in the complement of the second male (compare Figs. 7 and 11). Whether or not the shorter partner has also undergone structural change is not known, since it is so similar in size and kinetochore position to several of the larger autosomes that its homologue in the second male cannot be identified. The random segregation of the X and the two unequal autosomes can be seen at anaphase I, and is again evident in the frequencies of the 4 types of metaphase II plates to which it gives rise.

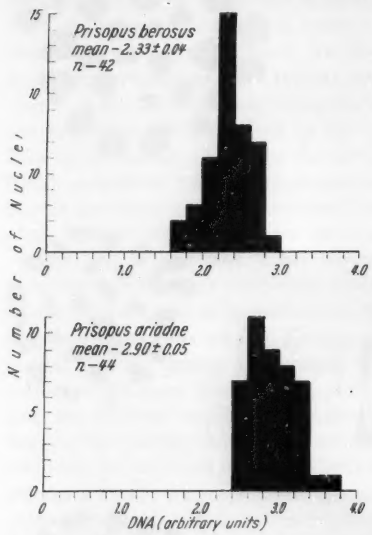


Fig. 14. DNA in spermatid nuclei of *P. berosus* and *P. ariadne* (Feulgen cytophotometry); *mean* = average nuclear content of DNA (in arbitrary units), with Standard Error; *n* = number of nuclei measured

The X chromosome also shows structural diversity. In the male with the unequal autosomal pair, the mid region of the X tends to uncoil early in the confused stage; the X then appears bilobed, or more frequently, as two heterochromatic masses connected by a loosely coiled section. In the second male no such expansion occurs, nor is any differential coiling apparent at diakinesis or later in either specimen.

DNA values in Prisopus

To what extent the diverse chromosome complements of *P. ariadne* and *P. berosus* retain an equivalent amount of chromosomal material cannot be determined by visual comparison. Accordingly, to provide an index of total chromosomal content, cytophotometric measurements were made of the relative amount of DNA per nucleus in the two species. The results are shown in Fig. 14, as frequency distribution diagrams of the DNA content of spermatid nuclei. The DNA value of *P. ariadne* is significantly higher than that of *P.*

berosus: the ratio of the means is 1.4. The differentiation of the two complements has thus clearly involved the loss or addition of chromosomal material.

Discussion

1. The Neo XY sex chromosome mechanism

The transformation of the sex chromosomes from the XO ♂ — XX ♀ system characteristic of phasmids to Neo X, Neo Y status, found in *Prisopus ariadne* is not a unique event in the history of the group. It is known in three other species of widely different provenience: *Isagoras schraderi* REHN tribe *Prexaspeini*, subfamily *Pseudophasminae*, from Panama (HUGHES-SCHRADER 1947b); *Acanthoderus inermis* (REDT.) of the subfamily *Pygirrhynchinae*, from Brazil (PIZA 1950); and *Bacillus libanica* UVAROV, subfamily *Bacillinae*, from Israel, and endemic to the Near East (WAHRMAN, pers. com.). An independent evolutionary origin in each of the groups represented thus appears certain on the basis both of taxonomy and distribution. Indeed, in *Isagoras* and *Acanthoderus*, as in *Prisopus*, congeneric species are known which retain the ancestral XO—XX system.

The origin of these Neo XY mechanisms can be ascribed with a high degree of probability to a translocation between the single ancestral X and an autosome giving rise to the Neo X, while the homologous autosome thus restricted to the male line became the Neo Y. The heterochromatic arm of the Neo X retains the meiotic behavior of the original X, while the euchromatic nature of the pairing segments of Neo X and Neo Y attests their autosomal origin. The evolutionary significance of the heterochromatic arm of the Neo Y, found alike in *Prisopus ariadne*, *Isagoras schraderi* and *Bacillus libanica*, is less clear. Progressive heterochromatinization in the originally euchromatic Neo Y following its restriction to the male is inherently probable. Good evidence of such a process is afforded, for example, by the structural changes in the Neo Y among mantids carrying the monophyletic Neo X^1X^2Y sex chromosome mechanism. Alternatively, the heterochromatic arm of the Neo Y might also be derived in part or in whole from a more or less inert portion of the original X by a second translocation. The latter origin seems probable in the case of *Isagoras schraderi*, for here the heterochromatic arms of the Neo X and Neo Y are jointly polarized in the bouquet stage of meiotic prophase, precisely as are the two arms of the original X during its conflexion and polarization. In *Prisopus ariadne*, however, the heterochromatic arms of the two sex chromosomes are separately polarized and only rarely, and then transiently do they adhere together. This suggests a difference in the physical properties

of the heterochromatin of Neo X and Neo Y which in turn may reflect a difference in origin. Heterochromatinization in the Neo Y itself thus seems the more probable in *Prisopus ariadne*.

2. Evolutionary relationship of Prisopus karyotypes

An inverse relation between size and number of chromosomes is at once apparent in comparing the karyotypes of *Prisopus ariadne* and *P. berosus*. A direct Robertsonian relation is precluded by the presence in the *berosus* complement of at least 16 pairs of mediokinetic chromosomes. If whole arm transfers, involving breakage or fusion of kinetochores have occurred, the evidence has been masked by subsequent structural changes. The two complements do, however, afford evidence that other types of structural rearrangements have been involved in the differentiations of *Prisopus* karyotypes: translocation between sex chromosome and autosome is shown by the Neo XY of *ariadne*; interautosomal transpositions by the heteromorphic autosomes of *berosus*; and duplications or deletions are indicated by the difference between the two species in the nuclear content of DNA in male germ cells. The available evidence thus points to the accumulation of structural rearrangements as the major factor in the evolution of *Prisopus* karyotypes. A wider sampling of the included forms may well permit a more precise analysis of interspecific relations.

3. Cytotaxonomy of phasmids

The cytology of bisexual phasmids is marked by the retention in all subgroups studied of the characteristic pattern of male meiosis exemplified by *Prisopus*. But the most striking cytological feature of the group is the diversity in chromosome number and morphology which appears repeatedly even among closely related species and is again evident at both the subfamily and family level. Although the chromosome numbers of several obligatorily parthenogenetic races and species strongly suggest a polyploid relation to conspecific or congeneric diploid forms (CAPPE DE BAILLON, FAVRELLE and DE VICHET 1938), there is as yet no evidence that polyploidy has been involved in the evolution of the bisexual species. In the latter, practically all intermediate steps between the highest and lowest numbers known are represented by one or more species, and the frequency distribution of species relative to chromosome number shows no peaks at multiple values. Even in those cases in which the chromosome number of one species is precisely doubled in a related form, the morphology of the chromosomes in the two complements precludes a simple polyploid relationship (FAVRELLE 1934; CAPPE DE BAILLON, FAVRELLE and DE VICHET 1938). As in the *Prisopini*, the

available evidence points to the accumulation of diverse structural rearrangements as the major factor in karyotype evolution among bisexual phasmids in general.

As to the nature of these structural changes, some information is available. Whole arm transfers have not been demonstrated; their absence was noted already in 1934 by FAVRELLE in the first comparative study of spermatogenesis in normally bisexual forms. Later work extends the number of cytologically known species to include representatives of 10 of the 19 subfamilies currently recognized; it confirms FAVRELLE's conclusions, and shows that the same kind of structural changes evident in *Prisopus* have occurred repeatedly in the evolution of the group. As examples may be cited the four cases of X-autosome translocation, all of independent origin, discussed above, and the presence of unequal autosomal pairs in five species of two subfamilies (CAPPE DE BAILLON and DE VICHET 1940; HUGHES-SCHRADER 1947b).

The general features of the ancestral phasmid karyotype may be inferred from what is now known of the chromosomes of the more primitive living species. While the evolutionary relation between the two constituent families of the *Phasmatodea* is not clear, within each family certain subgroups stand out as persistently primitive. Of the *Phylliidae* (= *Areolatae* REDT.) the *Phylliinae* appear second only to the *Prisopini* in this respect (GÜNTHER 1953). The chromosomes of the one species studied, *Phyllium bioculatum*, number 33, of which at least two pairs of autosomes are mediokinetic (FAVRELLE 1934). In the family *Phasmidae* (= *Anareolatae* REDT.) the *Phasminae* rank among the most primitive, and again a single species is cytologically known. This is *Clitumnus extradentatus*, $2n♂ = 37$, of which only one pair of the autosomes is mediokinetic (BERGERARD 1958). Certain of the *Phibalosominae* also retain primitive features, and here cytological data are available on 3 species: *Aplopus mayeri* (JORDAN 1908) and *Bostra sp.* (HUGHES-SCHRADER 1947a) each with 35, and the more specialized *Phibalosoma phyllium* with 41 (PIZA 1950). Most of the autosomes of *Bostra* are mediokinetic, as are certain pairs in the other two complements, to judge solely from the published figures. In all the above species the X is a relatively large mediokinetic element. The foregoing data show a narrow range in chromosome number—from 33 to 37—to predominate among these primitive phasmids. They suggest an ancestral karyotype numbering approximately 35 ($2n♂$), comprising a large mediokinetic X and relatively small autosomes, and with a basic arm number of about 40. On this hypothesis the existence in the primitive *Prisopini* of karyotypes so diverse as those of *P. ariadne* with 28 and *P. berosus* with 49 chromosomes is explicable on the assumption that both are derived types, evolutionary change

in chromosome number having proceeded in both directions from the ancestral norm. The close similarity of the two species in gross morphology further suggests that their differentiation has occurred in relatively recent time. We may therefore expect to find within the genus or the tribe species with complements approximating 35 in number and retaining the primitive features cited above.

Extensive structural reorganization of the ancestral karyotype, similar to that postulated for *Prisopus*, must have occurred repeatedly in the evolution of the phasmids to give rise to the wide diversity in chromosome complements now characterizing families, subfamilies and genera. It follows naturally that phylogenetically significant correlations between the cytological characters and the taxonomic categories are difficult to establish. Taxonomically also, the phylogenetic relationship of the higher categories remains uncertain; it seems impossible to correlate the data of taxonomy as now organized with those of geographical distribution. Clarification of the many still baffling aspects of phasmid evolution can only come through inter-relating pertinent evidence from all fields. Further comparative cytological studies should certainly be directed to the phasmid taxa exhibiting anomalous geographic distribution.

Summary

A wide diversity in chromosome complement is found in two species of phasmids of the primitive group *Prisopini—Prisopus ariadne* HEBARD and *Prisopus berosus* WESTWOOD.

P. ariadne has a diploid male complement of 28, comprising 13 pairs of relatively large mediokinetic autosomes and Neo XY sex chromosomes.

P. berosus, 2n ♂ = 49, has relatively small autosomes most of which are mediokinetic, and retains the XO—XX sex mechanism. Chromosomal polymorphism in this species is suggested by the presence of an unequal pair of autosomes and a structural differentiation in the X in one of two males studied.

The relative amount of DNA per nucleus in male germ cells (Feulgen cytophotometry) shows a significant difference in total chromosomal content between the complements of the two species.

These data are discussed with reference to the cytotaxonomy of phasmids.

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Chromosoma (Berl.) 10, 278—300 (1959)

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LECANOID CHROMOSOME BEHAVIOR IN THREE MORE FAMILIES OF THE COCCOIDEA (HOMOPTERA)*

By

SPENCER W. BROWN

With 52 Figures in the Text

(Eingegangen am 27. Januar 1959)

The lecanoid system of chromosome behavior was first discovered by SCHRADER (1921) in a mealy bug, *Planococcus (= Pseudococcus) citri* (RISSO). Further studies by SCHRADER and others showed that this system occurred in several families of coccids and led to its accurate characterization (see HUGHES-SCHRADER 1948). The males have the same chromosome number as the females but differ from them markedly in the deportment of the chromosomes. In the males, one haploid set becomes heterochromatic at an early embryonic stage, remains so throughout development, but is eliminated from the genetic continuum at spermatogenesis. Except in *Gossyparia*, to be considered later, the first meiotic division is equational for all chromosomes; during the second division, the heterochromatic set separates from the euchromatic haploid set. Of the four products of meiosis, only the two euchromatic derivatives form sperm; the heterochromatic products degenerate during spermiogenesis.

The present report provides descriptions of chromosome behavior at spermatogenesis in one species each of three families not previously studied cytologically. Since this work was undertaken largely for comparative purposes, further consideration of prior cytological and taxonomic studies will be given in the discussion in conjunction with the conclusions from the present findings.

It should be emphasized here, however, that there are several reasons for continued interest in such comparative studies. The lecanoid system enables a diploid organism to breed as a haploid (SCHRADER and HUGHES-SCHRADER 1931); comparisons of lecanoid systems in various taxa may be expected to show how much variation this system can undergo yet maintain the same genetic outcome.

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The definition of the major subdivisions of the *Coccoïdea* is at present the subject of considerable disagreement (FERRIS 1957). The lecanoid system of chromosome behavior is, itself, unique, and should provide an invaluable diagnostic criterion; there seems little doubt that those groups which possess it share a common ancestry.

The most specialized family of coccids, the armored scales or *Diaspididae*, presumably have their closest affinities among the families under consideration here. In the armored scales, the males become haploid by eliminating the paternal chromosome set during early embryogeny, near the time when heterochromatization usually begins in the lecanoid system (BROWN and BENNETT 1957). Investigation of groups believed to be closely allied to the armored scales is essential for an understanding of this relationship between the origin of a major category and the evolution of a genetic system.

Materials and Methods

Two solutions were used for fixing and storing: Carnoy I (1 part glacial acetic acid, 3 parts absolute alcohol, by volume) and the modification of Carnoy II developed by BRADLEY (1948) for a study of plant embryo sacs (1 part glacial acetic acid, 3 parts absolute alcohol, and 4 parts chloroform, by volume).

Collections of *Cerococcus quercus* COMSTOCK were obtained during July and August 1957, from a stand of scrub oaks, *Quercus dumosa* NUTT: at Cajon Pass, about 29 miles north of San Bernardino, California, along U. S. highway No. 66—395. Females of the preceding generation and young females were fixed in the Bradley-Carnoy solution; young males, in either this solution or Carnoy I.

Material of *Tachardiella sp.* was collected from the tarbush, *Flourensia cernua* DC., near Portal, Cochise County, Arizona, during June and July, 1958. These insects were readily fixed by immersing the twigs on which they were borne directly in large volumes of the Bradley-Carnoy mixture which dissolved the encrusting lac. After 6 to 12 hours the young males and females were brushed from the twigs and transferred to Carnoy I for storage; the older females were no longer suitable for cytological analysis.

Two generous collections of young males and females of *Conchaspis lepagei* HEMPEL were fixed and forwarded by Dr. JOSÉ PINTO DA FONSECA from São Paulo, Brazil, in March and July, 1958, the latter from *Astrocaryum agrii*.

Aceto-carmine squashes were made directly from either fixing solution. If the material had been stored for several weeks or longer, the staining was somewhat improved by gentle heating over a spirit lamp immediately before squashing. For the Feulgen procedure with *Tachardiella* males, optimum hydrolysis in 1 N HCL at 60° C. proved to be but 4 minutes; after staining and repeated rinsing in sulphite water, the material was squashed in 45% acetic acid.

The semi-diagrammatic sketches and photographs were made from fresh aceto-carmine squashes. The original negatives were exposed at a magnification of 1140×, and enlarged to about 4150× for composition of the plates; the final magnification on printing is about 3320×.

Observations

Cerococcus quercus COMSTOCK

C. quercus is a member of the *Asterolecaniidae*, a family recognized by FERRIS (1955) as a rather diverse assemblage, and considered by BALACHOWSKY (1942) as separable into the major subfamily and the *Cerococcinae*. The species under consideration should thus be regarded as belonging to a rather atypical genus of a motley assemblage.

The older females retained a few unlaid eggs showing various stages of embryonic development. The embryos were of two classes, those with and those without heteropycnotic bodies in the resting nuclei. Although the definite stage in embryonic development at which the males begin to show heteropycnosis was not precisely determinable, the observations did indicate that this process must occur in early embryogeny. Comparison with the young males and females showed that the heteropycnotic structures, were, as to be expected, characteristic of the males.

The sex of the young individuals could be readily determined both externally and from internal anatomy. In the young females, division figures showing 18 chromosomes were found in egg sheath cells; there was no evidence of heteropycnotic bodies in this or other tissues. By contrast, the young males showed striking heteropycnotic structures in the resting nuclei. Somatic division figures in the male (Fig. 1) also demonstrated 18 chromosomes.

Spermatogenesis

Stages earlier than mid-prophase were not studied. At mid-prophase, the meiocyte nuclei display a heteropycnotic blob obviously composed of clumped chromosomes and 9 lightly stained but fairly well contracted euchromatic chromosomes (Fig. 2). The heterochromatic material lies at one side of the nucleus and the euchromatic chromosomes are distributed rather uniformly in the remainder of the space. During the mid- to late prophase period, the heteropycnotic element becomes resolved into 9 discrete chromosomes which remain closely associated; the euchromatic chromosomes condense further until they soon stain as darkly as the members of the heterochromatic set. While completing the condensation process, the chromosomes are scattered but begin to approach the heterochromatic group at the end of prophase.

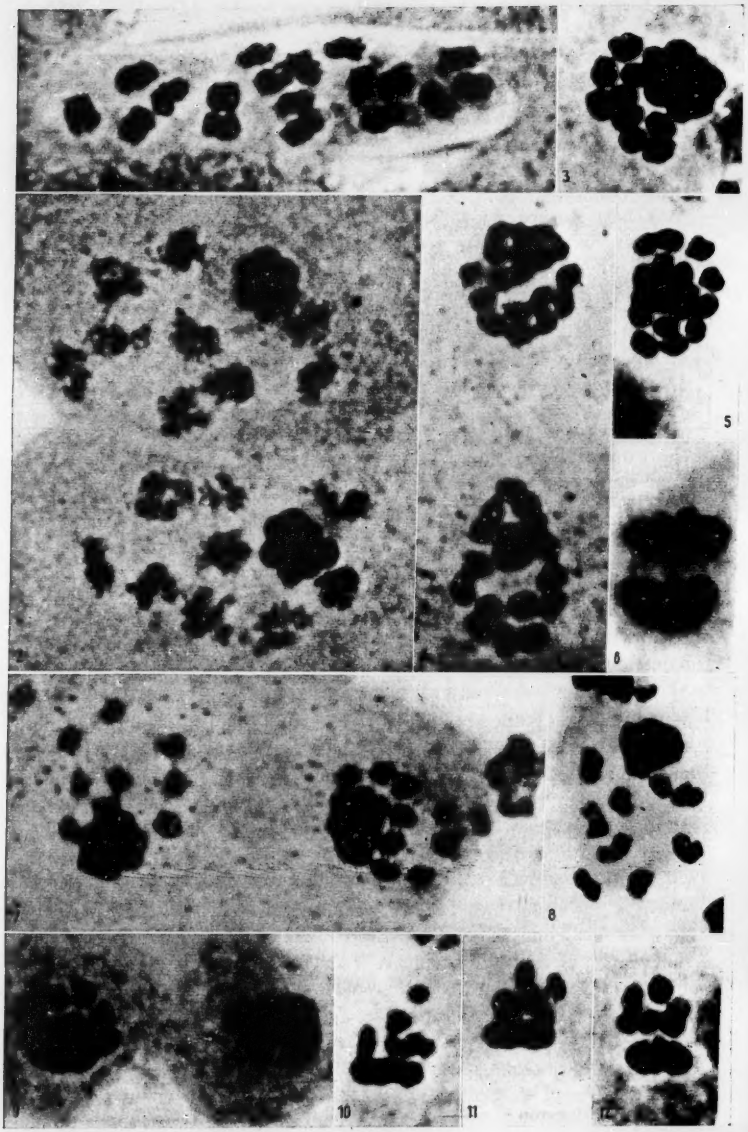
The distribution of the chromosomes on the metaphase plate is of special interest. The 9 heterochromatic chromosomes form a compact group while the 9 euchromatic chromosomes are more loosely deployed around it, sometimes in a single line, but often grouped to one side

(Fig. 3 and 4). Sometimes a niche is present in the compact arrangement of the heterochromatic chromosomes and in this a euchromatic member may then be found (Fig. 5). The metaphase pattern may be readily understood as a consequence of the prophase distribution which, as already noted, results from the position of the heteropycnotic body at one side of the nucleus.

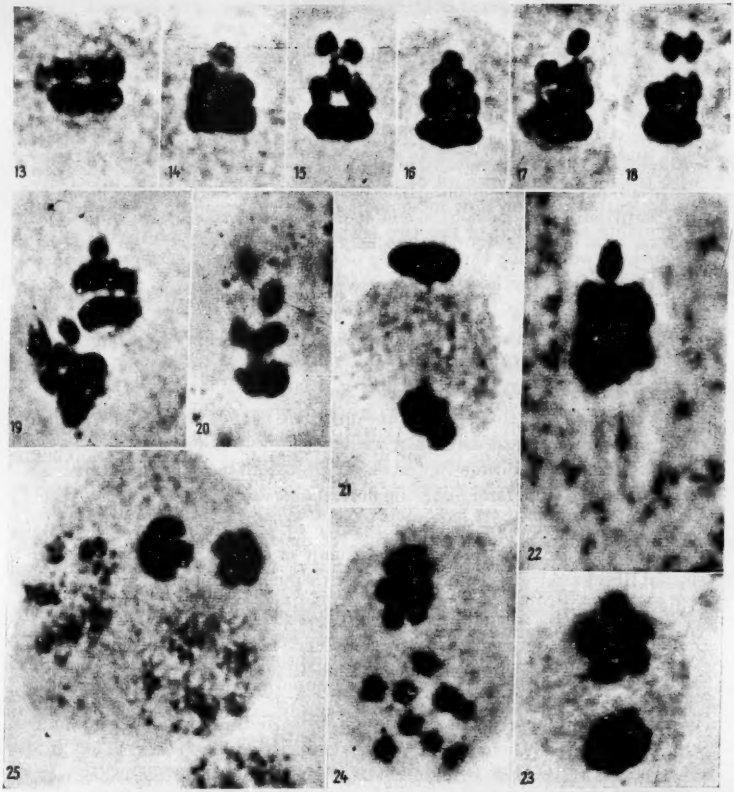
Anaphase I separations show little but the confinement of the daughter groups to two restricted plates which remain parallel to each other while moving apart (Fig. 6). An ephemeral aspect of telophase I is reminiscent of the late prophase condition with the euchromatic daughter chromosomes distributed around a tightly associated heterochromatic daughter group. Because of the nature of the anaphase separation, all are precisely aligned in a very flat plane (Fig. 7).

Telophase I, interkinesis (Fig. 8) and prophase II are all apparently of short duration involving mainly a slight loosening of the euchromatic chromosomes and their random scattering. It was not possible in the squashed material to follow the establishment or breakdown of a nuclear membrane during this period. During late prophase II, the scattered euchromatic chromosomes move toward the heterochromatic group and become loosely appressed to one side of it (Fig. 9—12). During prophase II, as in prophase I, the heterochromatic group has remained to one side of the region occupied by the chromosomes. Chromosome congression at late prophase II differs from that at prophase I in that in the latter the euchromatic chromosomes group themselves around the heterochromatic clump so that the entire complement forms a flat plate while in the former the chromosomes tend to form two separate plates with their flat sides close together. The euchromatic group seldom, however, forms a completely flattened plate (Fig. 14). Metaphase II may be defined as the stage at which the euchromatic chromosomes show the narrowest profile of flattening against the heterochromatic chromosomes (Fig. 13). The two flattened groups do not usually appear to be in direct contact but seem to remain separated by a narrow clear zone (Fig. 12) which is cut across by strands or fibres (see below). The arrangement at second metaphase is thus a direct anticipation of the separation of the hetero- from the euchromatic chromosomes at second anaphase.

Anaphase II is the critical stage in the lecanoid system of chromosome behavior because it is at this juncture that the two different sorts of chromosomes are segregated to opposite ends of the cell. As has just been noted, however, at the onset of anaphase II, the two different chromosome groups are already segregated on opposite sides of the



Figs. 1—25. *Cerococcus quercus*. Fig. 1. Somatic division figure in male, 18 chromosomes. Figs. 2—25. Spermatogenesis. Fig. 2. Late Prophase I; two cells, each with 9 euchromatic chromosomes and



dense group of heterochromatic chromosomes. Fig. 3. Metaphase I; 9 euchromatic chromosomes, left, and 9 heterochromatic chromosomes in a group, right. Fig. 4. Metaphase I; two cells, the heterochromatic group uppermost in each. Fig. 5. Metaphase I; euchromatic chromosomes in a single line around heterochromatic group except for member at 5 o'clock. Fig. 6. Anaphase I; semilateral view. Fig. 7. Telophase I; two cells each with 9 euchromatic chromosomes and the heterochromatic group (chromosome at 3 o'clock in cell at right belongs to adjacent figure). Fig. 8. Interkinesis; 9 euchromatic chromosomes and the heterochromatic group; note the clearly split chromosomes at 3 o'clock. Fig. 9. Prophase II; two cells in each of which the euchromatic chromosomes have begun to gather near the heterochromatic group. Fig. 10. Prophase II; the heterochromatic group is lowermost and the euchromatic chromosomes have begun formation of the double plate; lateral view. Fig. 11. Prophase II; heterochromatic group lowermost, euchromatic chromosomes gathered together on one side of it. Fig. 12. Prophase II; as Fig. 11, semilateral view showing clear zone between euchromatic and heterochromatic groups. Fig. 13. Metaphase II; complete double plate formation, euchromatic group above. Fig. 14. Metaphase II; incomplete double plate formation, euchromatic group, above. Figs. 15—19. Early Anaphase II; formation of fibrils, orientation of chromosomes and slow movement of the euchromatic groups, above. Fig. 20. Anaphase II; interzonals between the lowermost members of the euchromatic group and the heterochromatic group, below. Fig. 21. Telophase II; interzonals between the euchromatic (above) and heterochromatic derivatives. Fig. 22. Anaphase II; half-spindle characteristic of the heterochromatic groups, below; the chromosomes here are partially obscured by overlying material. Fig. 23. Early Telophase II; euchromatic group, above, is clumped. Fig. 24. Late Telophase II; 9 chromosomes apparent in euchromatic group, below; note split chromosome at 10 o'clock. Fig. 25. Quadrinucleate spermatid; two heterochromatic, above, and two euchromatic derivatives

metaphase plate and each is facing the end of the cell toward which it will move. Chromosome movement commences with the slow progress of the now somewhat agglutinated euchromatic members toward the adjacent pole (Fig. 15—19). Usually one or two chromosomes are in the lead, and the division figures as a whole acquire a triangular appearance with the heterochromatic set at the base (note especially Fig. 16). Only infrequently does the slow movement of the euchromatic set effectuate a complete separation of the two sorts of chromosomes. Shortly after the one or two leading chromosomes of the euchromatic set have reached the polar region, the heterochromatic set moves rapidly to the opposite end of the cell (Fig. 20).

The second division figure has been characterized as having but a single pole to which the heterochromatic elements migrated while the euchromatic chromosomes remained passive, displaying at most a few, weak chromosomal fibres. More recently RIS has reinterpreted the lecanoid kinetic apparatus as bipolar in structure but essentially monopolar in function (see HUGHES-SCHRADER 1948). Anaphase II of *C. quercus* seems readily understandable in accordance with RIS' interpretation.

In the squashes, with either regular or phase contrast optics, the actual fibres are observable with difficulty. Only a few short chromosomal fibres are to be seen connected with the euchromatic chromosomes (Fig. 15 and 19); when they can be identified, those leading from the heterochromatic group can be seen to form a typical, acuminate half-spindle (Fig. 22). Fibrils between the euchromatic and heterochromatic sets first become noticeable at late prophase and seem to form the basis for the interzonals which first appear definitely as such at mid-anaphase (Fig. 20) and are maintained until telophase (Fig. 21).

Shortly after the completion of anaphase II movement, the two different chromosome sets resemble each other more closely than at any other time during meiosis (Fig. 23). Nonetheless, the differentiation that is maintained is sufficient to show that it is the euchromatic set which forms the resting nucleus from which the sperm originate (Fig. 24 and 25). Nine chromosomes can be counted in both types of nuclei at telophase II; although those in the heterochromatic derivative are too clumped for photographic demonstration, such is not the case with the other set (Fig. 24).

The first cytokinesis occurs after telophase I; the second is a transient separation after telophase II, at the end of which all four cells recombine to form a quadrinucleate spermatid. Two sperm are formed from each spermatocyte, from the two nuclei containing the euchromatic chromosomes. Later stages of spermiogenesis were not followed in detail; the nuclei containing the heterochromatic chromo-

somes disappear as stainable, identifiable objects shortly before the residual protoplasm of the cyst is lost.

In summary of the observations on spermatogenesis in *C. quercus*, it may be stated that the chromosome behavior here is typically lecanoid. Eighteen chromosomes, 9 of which are heterochromatic, 9 euchromatic, divide equationally at the first division; during the second division, the 9 heterochromatic chromosomes separate from the 9 euchromatic members to give four nuclei of which only the two euchromatic derivatives will form sperm.

Symbionts

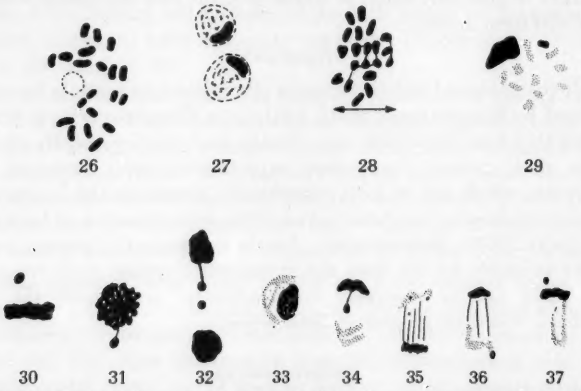
Microorganisms identified from *in vitro* culture as bacteria have been reported by MAHDIHASSAN (1933, 1951) from *Cerococcus ornatus* GREEN, presumably from the female sex. It may be noted here briefly that the males of *C. quercus* also possess mycetocytes richly endowed with symbionts which are at least superficially similar to the bacteria-like organisms present in most but not all of the asterolecaniids so far studied (BUCHNER 1953). Unfortunately, female material of *C. quercus* was no longer available by the time the chromosome studies were completed and could not be examined for symbionts; presumably the same organism would be present in both sexes.

Tachardiella sp.

This genus is a member of the *Lacciferidae*, the lac insects, a family recognized by both FERRIS (1955) and BALACHOWSKY (1942). The sexes could be differentiated both externally, by the enlarged protuberances on the young females, and internally. The resting nuclei of the males show the typical heteropycnotic body (Fig. 27) while those of the female do not. The chromosomes in all figures observed were quite small and usually too numerous and close together to make exact counting possible. A few division figures were clear enough to permit counts which are believed to be accurate (Fig. 26 and 28), and mitoses in both somatic and germinal tissues in both males and females showed 20 chromosomes. The spermatogenic divisions of *Tachardiella sp.* are typically lecanoid in general aspect but the exact numbers of heterochromatic and euchromatic chromosomes could not be followed through the various stages. The only counts which have been made with any degree of certainty are those at late prophase I at which time 10 euchromatic chromosomes are scattered about while the remainder, presumably 10 heterochromatic chromosomes, form the heteropycnotic mass on one side of the nuclear region (Fig. 29).

In addition to the two sorts of chromosomes there is present also a third element which will be referred to, as non-committally as possible,

as an accessory structure. This element is identifiable throughout much of the course of spermatogenesis but has not been recognized during mitotic divisions in either sex. The accessory structure stains brilliantly in the aceto-carmine squashes where it appears most clearly and characteristically at metaphase I. However, it could not be identified at this stage following a Feulgen reaction, although the chromosomes were stained intensely.



Figs. 26—37. *Tachardiella sp.* Fig. 26. Somatic figure from young female. Fig. 27. Two nuclei from young male showing typical lecanoid heteropycnotic body. Fig. 28. Spermatogonial division, arrow = 4 μ. Figs. 29—37. Spermatogenesis. Fig. 29. Prophase I; 10 euchromatic chromosomes; the clump of heterochromatic chromosomes, 11 o'clock; the accessory structure, 12 o'clock. Fig. 30. Metaphase I; lateral view with unattached accessory structure in poleward position (12 o'clock). Fig. 31. Metaphase I; polar view with attached accessory structure in lateral position (6 o'clock). Fig. 32. Telophase I; division of accessory structure, upper division product with exceptionally heavy attachment. Fig. 33. Interphase I or early Prophase I; heterochromatic group in dense clump at right; euchromatic chromosomes in a diffuse state on left. Fig. 34. Telophase II; accessory structure attached to heterochromatic group. Fig. 35. Telophase II; accessory structure fragmented, pieces associated with euchromatic group. Fig. 36. Telophase II; accessory structure associated with euchromatic group. Fig. 37. Anaphase II; accessory structure adjacent to heterochromatic group

At late prophase, the accessory structure is identifiable in aceto-carmine as a heavily stained element contrasting markedly with the euchromatic chromosomes (Fig. 29). It was not possible to determine whether it was originally an intra- or extranuclear body nor did the protoplasm immediately surrounding it appear to be differentiated.

At metaphase I, the chromosomes are tightly grouped in a flat plate; they cannot be counted nor can the euchromatic members be distinguished from the heterochromatic. In about 50% of the cases, the accessory structure is attached to the metaphase group by a thin fibril (Fig. 31 and 38). Lateral views show that it is most frequently

situated between the chromosomes and one pole, but it may also occur to one side of the metaphase plate (Fig. 30 and 38).

The chromosomes separate at anaphase as two flat daughter plates; the accessory structure lags behind and undergoes division during telophase, at times retaining its connection to the chromosomes (Fig. 32). From late telophase I to late prophase II, the two types of chromosomes are again distinguishable; the euchromatic members form a diffuse crescent around the compact heterochromatic group (Fig. 33). At metaphase II, the two types of chromosomes appear as closely approximated groups in which no detail can be seen.

With the onset of anaphase II, the separation of the diffuse euchromatic chromosomes from the heterochromatic members is elegantly displayed. Interzonal fibres are frequently to be observed, but no other parts of the achromatic figure have been identified. The accessory structure shows variable behavior during the second ana- and telophases (Fig. 34—37). At late telophase, it has frequently been found to be associated with the euchromatic group (Fig. 36). Yet the density of both types of nuclei, and especially of the heterochromatic derivative, during part of the telophase period, has precluded more certain determination of its fate. In addition fragmentation of the accessory structure (Fig. 35) complicates its identification.

Fig. 38. *Tachardiella sp.*, spermatogenesis. Metaphase I, two division figures with an unattached accessory structure (see text) at 12 o'clock in the upper figure and an attached accessory structure at 4 o'clock in the lower figure

Cytokinesis and spermiogenesis have not been followed in detail although numerous observations made at the later stages have shown again the typical lecanoid picture of large heteropycnotic bodies remaining in the cyst while the euchromatic derivatives form the sperm.

In conclusion, the observations just described testify to a lecanoid system of chromosome behavior in this species of *Tachardiella* even though the individual chromosomes are small and, for this and other reasons, cannot be followed through the spermatogenic stages as separate entities. The accessory structure does not seem to have an influence on the behavior of the chromosomes.

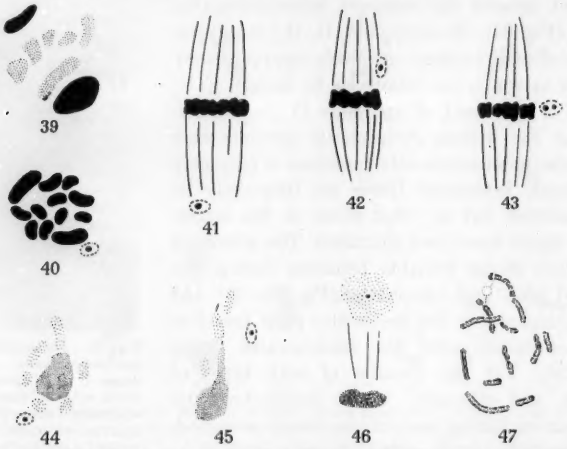
Conchaspis lepagei HEMPEL

The material of *C. lepagei*, kindly provided by Dr. JOSÉ PINTO DA FONSECA, consisted of young females, some of which were sufficiently advanced to contain young ovarioles, and young to adult males. It is

thus possible to be certain that again it is the male sex which shows the typical lecanoid heterochromatization while the female does not.

Twelve chromosomes are present at somatic divisions in the female (Fig. 47) and in the male.

At late prophase of spermatogenesis, the heterochromatic chromosomes are either all together in one clump (Fig. 48), or, frequently, one may be separated from the rest (Fig. 39). The separate heterochromatic

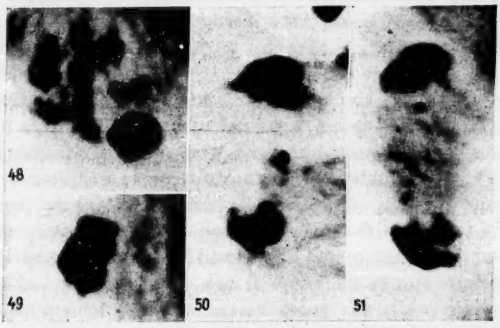


Figs. 39—47. *Conchaspis lepagei*. Figs. 39—46. Spermatogenesis. Fig. 39. Prophase I; 6 euchromatic chromosomes; with the exception of one member (11 o'clock) the heterochromatic chromosomes form a heteropycnotic body. Fig. 40. Prometaphase I; accessory structure at 5 o'clock. Fig. 41. Metaphase I; lateral view; accessory structure in lower polar zone. Fig. 42. Metaphase I; accessory structure at edge of spindle. Fig. 43. Metaphase I; divided accessory structure in the lateral position. Fig. 44. Telophase I; 6 euchromatic chromosomes surround the clump of heterochromatic chromosomes; accessory structure, below. Fig. 45. Anaphase II; euchromatic group, above; heterochromatic, below; not completely distinguishable; accessory structure on right. Fig. 46. Telophase II; euchromatic derivative, with accessory structure at center, above; heterochromatic derivative below. Fig. 47. Somatic division figure from young female

entities may represent any of the chromosomes which are distinguishable by size. The euchromatic chromosomes appear in the expected number of 6 (Fig. 39 and 48). At very late prophase, or prometaphase, the two types of chromosomes can no longer be distinguished as such (Fig. 40).

An accessory structure similar to that already described for *Tachardiella sp.* can be identified during the spermatogenic stages. No Feulgen tests were made of the *Conchaspis* material, however. The accessory structure in *Conchaspis* usually appears to be surrounded by a faint halo which is maintained during the division sequences but could not be captured in the photographs.

The accessory structure is identifiable at late prophase and appears near, but usually not attached to the metaphase groups (Fig. 40, 42, and 49); it may occur at or near one of the poles precociously (Fig. 41). Actual separation occurs, again, as in *Tachardiella sp.*, at telophase I; after the chromosomes have separated, the lagging accessory structure divides and the two products move toward opposite poles (Fig. 50 and 51). However, evidence of actual division is often apparent much earlier



Figs. 48—51. *Conchaspis lepagei*, spermatogenesis. Fig. 48. Prophase I; 6 euchromatic chromosomes and dense group of heterochromatic chromosomes. Fig. 49. Metaphase I; semilateral view, accessory structure at 2 o'clock. Fig. 50. Telophase I; accessory structure half way between the two daughter groups and undivided. Fig. 51. Telophase I; division products of accessory structure approaching daughter chromosome groups

(Fig. 43). The six euchromatic chromosomes are again differentially displayed at late telophase I, and the accessory structure is identifiable (Fig. 44), presumably now as a division product of the original. Second division stages were neither very clear nor abundant in the collections. Prophase stages appeared as a simple clumping to give a rather gross conglomeration from which the two types of chromosomes separated, at first gradually (Fig. 45), to display eventually at late telophase the striking differences between the euchromatic and heterochromatic nuclei (Fig. 46). The accessory structure is present as a large single granule (Fig. 46) or as several smaller granules. Subsequently, the euchromatic derivatives undergo spermiogenesis while the heterochromatic blobs remain for a long while unaltered.

Discussion

Cytological aspects

Of the three species considered in this report, only one, *Cerococcus quercus*, is of value for detailed cytological comparisons with other examples of lecanoid chromosome behavior. The other two species,

Conchaspis lepagei and *Tachardiella sp.*, both show the more general aspects of lecanoid behavior but in neither could the complete sequence be followed with satisfactory clarity.

In its chromosome behavior, *C. quercus* most closely resembles *Phenacoccus acericola* KING, the species analyzed by HUGHES-SCHRADER (1935) and later chosen by her (1948) to typify the lecanoid system. For a comparison of the situation in *P. acericola* with those described by SCHRADER (1921, 1923) for *Pseudococcus citri*, *P. nipae*, and *P. maritimus* and by THOMSEN (1927) for *Lecanium hesperidium* and *L. hemisphaericum*, the reader should consult HUGHES-SCHRADER'S (1948) review.

Centrioles were not identifiable during either spermatogenic division in *C. quercus* but were clearly apparent in *P. acericola*. This difference may perhaps be due to technical difficulties, since the squashing method may often fail to demonstrate the achromatic figure properly.

At second division, a much more definite metaphase condition is achieved in *C. quercus* than in *P. acericola*. In the latter species, there are no prophase II stages, and anaphase II directly follows telophase I; the heterochromatic group forms a monopolar spindle, and moves to the single pole while the euchromatic members remain passive. In *C. quercus* the second division does not proceed so abruptly; as already described in considerable detail, prophase II movement leads to the formation of a double metaphase plate, with the euchromatic and heterochromatic members each forming one layer. Early anaphase in *C. quercus* is characterized by a slow progression of the euchromatic members to form a pyramidal arrangement with the peak near one end of the cell. Although they move only a short distance and do not separate from the heterochromatic group, the euchromatic members do show orientation in respect to the axis of movement and evidence of stress. Except for a very clear zone between the two groups, actual separation of the two sorts of chromosomes occurs only on movement of the heterochromatic group.

Because of its phylogenetic position (see next section), *C. quercus* would be suspected of having a more highly specialized spermatogenesis than *P. acericola* and other species of the primitive *Pseudococcidae*. Chromosome behavior in *C. quercus* might appear, superficially, to be more like normal meiosis than *P. acericola* because of the second prophase and metaphase stages and of some movement of the euchromatic members. However, it seems more likely that the formation of such a complex structure as a double metaphase plate, and the prerequisite prophase movements would represent secondary specializations.

The observations on *C. quercus* tend to support HUGHES-SCHRADER'S (1948) contention that chromosome behavior at second division in the

lecanoids is genetically determined and "not simply a mechanical result of asynchrony". Thus the same net result is achieved whether the euchromatic chromosomes remain completely passive as in *P. acericola* and others, or display precocious orientation and movement as in *C. quercus*. Although the differential behavior of the two different sets undoubtedly has its origin in their differential nature, the chromosomes are performing in a milieu which dictates how this differential nature is to be exploited. The relationship between behavior and any visible cytological difference is undoubtedly not a simple one.

The chromosomes of *C. quercus* are sufficiently large to demonstrate quite clearly that the chromosomes entering the sperm are, at least, double structures (Fig. 8 and 24). Although apparently not previously reported for the lecanoids, a similar double structure must be true for other coccids, including certain margaroid types in which the subdivision to half-chromatids is already clearly demonstrable at the first meiotic metaphase (HUGHES-SCHRADER 1948).

In *Tachardiella sp.* and *Conchaspis lepagei* the chromosomes did not present a definitive picture but the cytology was adequate for demonstrating characteristic features of the lecanoid system, the segregation of the two types of chromosomes at second division and formation of sperm only from the euchromatic group. Of special interest is the presence in both species of an accessory structure apparently not otherwise observed in the coccids. The accessory structure seems to play no part in the spermatogenic divisions themselves; it may be concerned with sperm formation or it may be centriolar material which becomes active, after fertilization, during the cleavage divisions. Since it is not stained after the Feulgen procedure, it is doubtful if the accessory structure is chromosomal in nature or an immediate derivative thereof.

Mention of SCHRADER'S (1929) studies of spermatogenesis in *Gossyparia spuria* (GEOFFROY) [now = *Eriococcus spurius* (MODEER)] (FERRIS 1955) has been saved until last since it represents to date the only fundamental modification yet discovered in a lecanoid system. In this species, the 14 heterochromatic chromosomes form 7 bivalents at the first meiotic division while the 14 euchromatic chromosomes remain unpaired. The result of the first anaphase separation is 21 chromosomes at each pole, presumably 7 heterochromatic and 14 euchromatic. During the second division, the euchromatic and heterochromatic groups separate in typical lecanoid fashion. HUGHES-SCHRADER (1948) has discussed two suggested explanations of the behavior in *Gossyparia*, one based on a permanent separation of the chromatids, and the other on tetraploidy, as proposed by WHITE (1945). It may only be noted at this point that as far as the genetic continuum is concerned, the hetero-

chromatic chromosomes would seem to be quite free to vary in their behavior as long as such changes do not prevent segregation of the euchromatic set and subsequent sperm formation by it. In this regard it is remarkable how little variation has been found in the lecanoid system among the different families in which it occurs.

Phylogenetic considerations

The coccids have been divided into about 15 families. There is little disagreement about four of these. The *Margarodidae* and the *Ortheziidae* obviously constitute an independent line of development from primitive forms. The *Diaspididae* and *Phoenicococcidae* are specialized groups, at the terminus of an evolutionary progression involving some of the other families. The problems to be considered are twofold: on the one hand, the relationships among these other families, and, on the other, an attempt to disclose which is most closely allied to the *Diaspididae* and *Phoenicococcidae*.

The various taxa and their placement by the two foremost authorities in the field, BALACHOWSKY (1942) and FERRIS (1957) are cited in the Table (p. 294/95). The essential disagreement is in regard to those families which BALACHOWSKY lumps together in the section *Lecanoïdae* which to FERRIS is "too reminiscent of the subfamily *Dactylopinae* of the FERNALD Catalogue which long stood as a 'catch all' — a veritable waste basket — for the reception of all sorts of things and a cover for lack of knowledge". More recently BALACHOWSKY (1948) demoted the major subdivisions to families, and the families to subfamilies; his classification of 1942 will be used here, however, for comparison with that of FERRIS (l. c.).

Of the 12 taxa in the controversial assemblages, seven have now been demonstrated to have a lecanoid system of chromosome behavior while an eighth, the *Apiomorphidae*, has heterochromatization in some of the embryos (BUCHNER 1957b), and presumably a lecanoid system or modification. The lecanoid system is unique, no other like it being known for the living world. With the exception of the derivative diaspidid systems, to be considered below, the closest approach is to be found in the dipteran genus *Sciara* (METZ 1938) but the differences are almost as numerous as the similarities. Thus the presence of a lecanoid system is of especially great significance in indicating a common origin; BALACHOWSKY seems justified in placing diverse families together in the lecanoid section.

Furthermore, evidence from cytology indicates that those families with a lecanoid system of chromosome behavior must have originated from forms in which the lecanoid pattern of morphology was already

well-defined. It is quite clear that the basic chromosomal system for the coccids is the XX—XO mechanism which is present in some of the margarodids and also in a species of *Puto* of the relatively unspecialized *Pseudococcidae* (HUGHES-SCHRADER 1948). It thus seems obvious that the lecanoid system was derived, in a manner as yet unknown, from the XX—XO system (HUGHES-SCHRADER 1948), and that the first coccids with the lecanoid system of chromosome behavior were forms like the present pseudococcids. Thus the pattern of morphology identifiable as lecanoid had evolved prior to the appearance of the lecanoid system of chromosome behavior.

Those families which have not been studied in regard to chromosome behavior may now be briefly considered. The single species of the genus *Phenacoleachia* has been considered a unique form meriting establishment of a higher category (COCKERELL 1899, 1900; H. MORRISON and E. MORRISON 1922); although placing the *Phenacoleachiidae* in the *Lecanoïdae*, BALACHOWSKY (1942) noted resemblances to the *Margaroïdae*. The *Beesoniidae* are represented by but two species of the genus *Beesonia*, which, according to FERRIS (1950b) "is so utterly different from anything else that there seems nothing to do with it other than to assign it to a family by itself". Three families, the *Cylindrococcidae*, the *Apiomorphidae*, and the *Aclerdidae* have been included by one author or the other (see Table) in a family with a lecanoid chromosome system. BUCHNER (1957b) has observed heterochromatization in some embryos of the *Apiomorphidae*. These three families would be expected to possess the lecanoid system of chromosome behavior, but further cytological study is certainly warranted.

To FERRIS (1957), the genus *Capulinia* appeared to be unassignable to any family but perhaps to be related to the Australian genus *Opisthocelis*, and he (1955) had previously noted that "the Australian fauna of the *Coccoïdea* contains various forms which are in a similar position."

The genus *Stictococcus*, of economic importance in equatorial Africa, was left unplaced by FERRIS (1957); on the other hand, BALACHOWSKY (1942) recognized the family *Stictococcidae*, and noted a resemblance to the *Lecaniidae* on the basis of SILVESTRI's (1915) study of the males, and to the *Lacciferidae* because of impregnation of lac in the integuments. The genus is of current interest because of BUCHNER's (1954 and 1955a) recent discovery of the differential association of symbionts with the two sexes: only those eggs which are to develop into females are infected by the yeast-like microorganisms while those which are to become males remain free thereof. BUCHNER (1954) has attempted to generalize this phenomenon as an explanation for sex determination in other specialized coccids, such as the lecanoids, which lack the XX—XO sex determining mechanism. In all cases in which symbionts have been

Table. *Chromosome systems and sex-association of symbionts for the various families of the Coccoidea as classified by* BALACHOWSKY *(1942) and* FERRIS *(1957)*

Classification according to BALACHOWSKY: Major section / Family	Classification according to FERRIS: Major section / Family	Chromosome systems: Genus	Chromosome systems: Author	Chromosome systems: System	Sex-association of symbionts (+ present, 0 absent, — not reported): Genus	Sex-association of symbionts: Author	Sex-association of symbionts: Female	Sex-association of symbionts: Male
Margaroïdae	*Margarodi*							
1. *Margarodidae*	1. *Margarodidae*	several[1]	several[1]	several[1]	several	—[2]	+	+
2. *Ortheziidae*	2. *Ortheziidae*	*Orthezia*	BROWN[3]	—[3]	*Orthezia*	—[2]	+	—
Lecanoïdae								
	Eriococci							
3. *Eriococcidae*	3. *Eriococcidae*	*Gossyparia*[4]	SCHRADER 1929	lecanoid[4]	several	—[2]	+	—
4. (subfamily of 3)	4. *Pseudococcidae*	*Puto*	HUGHES-SCHRADER 1944	XX—XO	*Puto*[5]	BUCHNER 1955 b	+	+
		Pseudococcus	SCHRADER 1921, 1923	lecanoid	*Pseudococcus*	—[2]	+	+
		Phenacoccus	HUGHES-SCHRADER 1935	lecanoid	*Rastrococcus*	BUCHNER 1957 a	+	+
5. *Dactylopiidae*	5. *Dactylopiidae*	*Dactylopius*	HUGHES-SCHRADER 1948	lecanoid	*Coccus*[6]	—[2]	+	—
6. (subfamily of 9)	6. *Aclerdidae*	—	—	—	*Aclerda*	—[2]	+	—
7. *Cylindrococcidae*	7. (included in 3)	—	—	—	—	—	—	—
8. *Apiomorphidae*	8. (included in 3)	*Apiomorpha*	BUCHNER 1957 b	het. emb.[7]	*Apiomorpha*	BUCHNER 1957 b	0	0
9. *Lecaniidae*	9. *Coccidae*[8]	*Lecanium*	THOMSEN 1927	lecanoid	several	—[2]	+	—
10. *Asterolecaniidae*	10. *Asterolecaniidae*	*Cerococcus*	this report	lecanoid	several	—[2]	+	—
					Cerococcus	this report	—	+
	Lacciferi							
11. *Lacciferidae*	11. *Lacciferidae*	*Tachardiella*	this report	lecanoid	several	—[2]	+	—

	Beesonii							
12. —	12. *Beesoniidae*	—	—	—	—	—	—	—
13. *Stictococcidae*	13. (unplaced genus)	*Stictococcus*	BUCHNER 1954, 1955a	no het.[9]	*Stictococcus*	BUCHNER 1954, 1955a	+	0
14. *Phenacoleachiidae*	14. (unplaced genus)	—	—	—	—	—	—	—
	Diaspidi							
15. *Conchaspidae*	15. *Conchaspidae*	*Conchaspis*	this report	lecanoid	—	—	—	—
Diaspidoïdae								
16. *Phoenicococcidae*	16. *Phoenicococcidae*	*Phoenicococcus*	BROWN, unpubl.	het. emb.[7]	—	—	—	—
17. *Diaspididae*	17. *Diaspididae*	several	—[10]	several[10]	several	—[11]	+	+
18. unplaced genera: none	18. unplaced genera: 13, 14, *Capulinia*, *Opisthoscelis*							

[1] The review of HUGHES-SCHRADER (1948) should be consulted for further reference to detailed studies of the margarodids for which XX—XO, compound X, haplodiploid, and hermaphroditic, but no lecanoid or lecanoid-like systems have been reported.

[2] See review in BUCHNER (1953) on symbiosis in coccids and (1954) on symbionts in the male sex.

[3] Sex chromosomes were not identifiable in the one species reported by BROWN (in press); there was no evidence of lecanoid or similar type of chromosome behavior.

[4] See text for discussion of the *Gossyparia* modification of the lecanoid system; *Gossyparia spuria* (GEOFFROY) is now *Eriococcus spurius* (MODEER) (FERRIS 1957).

[5] Also species of *Puto* (= *Macrocerococcus*); FERRIS (1950a).

[6] (= *Dactylopius*).

[7] Typical lecanoid heterochromatization noted in some embryos, presumably male, and not in others, presumably female.

[8] (= *Lecaniidae*).

[9] Males lack the typical lecanoid heterochromatization.

[10] See discussion, this report, for consideration of the *Diaspididae*.

[11] See BUCHNER (1953, 1954). In addition, BROWN, unpubl., has made numerous observations of symbionts in male embryos.

studied in both sexes, and more have been added since BUCHNER'S (1954) report, they are present in both the males and females or absent in both sexes (Table), except in *Stictococcus* itself. He therefore suggested that the *quantity* of infecting symbionts, rather than their presence or absence, influences sex determination. This is not the place to argue the merits of BUCHNER'S suggestion in regard to sex determination, an idea which should provide a worthwhile stimulus for experimental analysis. His observations themselves, however, may be looked upon as additional characters differentiating the *Stictococcidae* from the specialized lecanoids, in regard to both the total absence of symbionts from the male sex, and the lack of heterochromatization in the males.

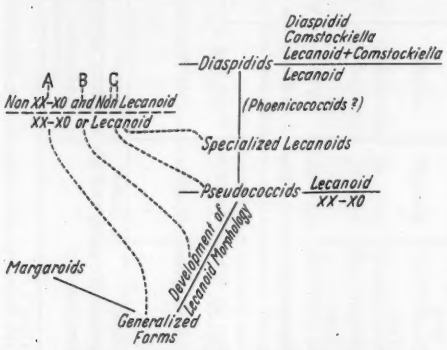


Fig. 52. Diagram illustrating known (solid lines) and hypothetical derivation (dotted lines) of systems of chromosome behavior in certain coccids. Some pseudococcids retain the primitive XX—XO mechanism; other pseudococcids and most, if not all, of the specialized lecanoids have the lecanoid system. Since the lecanoid system is still retained in certain armored scale insects in conjunction with the *Comstockiella* system, their immediate progenitors probably also had a lecanoid system. The dotted lines indicate possible derivations of forms with lesser to greater morphological affinities with the lecanoids but having unique forms of chromosome behavior. The evolution of the armored scales themselves may be looked upon as a "C" derivation with marked morphological changes

Viewed as a group, the unstudied genera or families are impressive as taxa of few species, rather well isolated from other groups. They may be relic types, the sole survivors of relatively unsuccessful lines of specialization, or more recent derivatives adapted to highly restrictive conditions. A chart has been prepared illustrating the presumed evolution of the coccids and showing the various possible origins of taxa which have some lecanoid morphological traits but do not have the lecanoid system of chromosome behavior, nor the primitive XX—XO mechanism (Fig. 52). Taxa of the A or B derivation would be expected to show systems of chromosome behavior without evidence of lecanoid ancestry; the C derivatives, on the other hand, would presumably have chromosome systems similar in some respects to the lecanoid as is the case with the *Diaspididae* to be discussed below. At present *Stictococcus* is the only likely candidate for A, B, or, especially, a C derivation; sufficient is known to rule out typical lecanoid heterochromatization in

the males (BUCHNER 1954 and 1955a), and the association of symbionts with only one sex, early in development, would seem to rule out an XX—XO mechanism.

On the basis of their morphology, some of the other genera may be expected to provide important evidence on the evolution of chromosome systems in the coccids. It should also be remembered that relatively few species or genera have been studied in any one family. That these specialized families are basically lecanoid in chromosome behavior seems unquestionable because of the wide range of this system. However, other genera within these families may have developed fundamental modifications.

In a diagram illustrating his views on the relationships of the various families of coccids, BALACHOWSKY (1942) places the *Stictococcidae*, *Lacciferidae*, and *Conchaspidae* together as an evolutionary line originating from the *Lecaniidae*. The occurrence of lac in both of the first two families has already been noted. The presence of the accessory structure at spermatogenesis, as described in the present report, is a characteristic of the *Lacciferidae* and *Conchaspidae* but has yet to be observed for any of the three systems of the *Diaspididae* or, apparently, for any other coccid. Considerable attention has been given to the *Conchaspidae* as possible allies of the *Diaspididae* because of the possession by the former of a leaf-like scale, and MAMET (1954) has recently noted similarities between the two families in the morphology of the tubular pores and in the structure of the males.

Origin of the armored scales

The armored scales represent the most highly specialized group of coccids, but their ancestry cannot be readily traced to any of the more primitive groups. The families suspected of being most closely allied are the *Asterolecaniidae* (BALACHOWSKY 1942), the *Conchaspidae* (MAMET 1954; FERRIS 1957) and especially the *Phoenicococcidae* (BALACHOWSKY 1942; FERRIS 1957). Of these three families, the first two are now known to possess a lecanoid system of chromosome behavior while observation of typical lecanoid heterochromatization in some embryos in *Phoenicococcus marlatti* COCKERELL (BROWN, unpubl.) indicates that probably this group also has a lecanoid or lecanoid-like system.

Among the armored scales, three types of chromosome behavior are now known. By far the most common is the diaspid system in which the males become haploid through the elimination of the paternal set of chromosomes at late cleavage, about the time at which heterochromatization appears in the lecanoid system (BROWN and BENNETT 1957; BROWN, unpubl.). A second type of chromosome behavior, the *Comstockiella* system, which may be so named for the genus in which

it was first described (BROWN 1957), also shows heterochromatization of one chromosome set in the male embryos; unlike the sequence in lecanoid spermatogenesis, however, in *Comstockiella*, all but two of the chromosomes form bivalents at late prophase of the single meiotic division, and then segregate to opposite poles, apparently at random. Of the two chromosomes showing differential behavior, one divides equationally, the other is eliminated during anaphase. Thus the heterochromatic set, except for the single member of the differential pair, is no longer subjected to elimination but its members have an equal chance with the euchromatic chromosomes of being transmitted to the next generation.

The third system in the armored scales is the lecanoid system itself which has not yet been found alone but only in company with the *Comstockiella* system (BROWN, unpub.). Possible phylogenetic relationships of the three systems cannot be discussed here. The finding of a lecanoid system in a diaspidid scale would seem to indicate that the immediate ancestors of the diaspidids probably also had the lecanoid system, and that cytological study of putative antecedent groups will probably provide little evidence of value in charting the phylogeny.

However, a study of a large number of the armored scales, themselves, should reveal some information about the evolutionary pattern within the family and this evidence may, by extrapolation, help to identify an ancestral type.

Summary

1. Chromosome behavior at spermatogenesis has been described for one species each of three families of coccids not previously studied in this regard: *Cerococcus quercus* COMSTOCK of the *Asterolecaniidae*, *Conchaspis lepagei* HEMPEL of the *Conchaspidae*, and *Tachardiella sp.* of the *Lacciferidae*. All three families belong to BALACHOWSKY's "lecanoid" section of the coccid superfamily, and the first two have been suggested, by various systematists, as close allies of the armored scales *(Diaspididae)*.

2. All three species showed lecanoid chromosome behavior, as originally described by the SCHRADERS, in that one haploid set of chromosomes is heterochromatic in the males and eliminated at spermatogenesis. The exceedingly clear cytology of *C. quercus* permitted a detailed study of the critical second division stages when the euchromatic and heterochromatic sets are separated. Although considerably more difficult cytologically, both the other species showed a typical lecanoid pattern of behavior; in addition, both showed an accessory structure, of unknown origin and function, which was present during the meiotic sequence.

3. The cytological and taxonomic implications of the results are considered, largely on a comparative basis, in the discussion. Special attention is given to the problem of the origin of the armored scales and also to BUCHNER'S recent suggestion of the possible influence of symbionts on sex determination.

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Dr. MONT CAZIER of the Southwestern Research Station of the American Museum of Natural History, Portal, Arizona, was responsible for suggesting the study of a locally available lac insect, *Tachardiella* sp. In addition, facilities for cytological research were utilized during the author's visit to the Research Station.

Dr. JOSÉ PINTO DA FONSECA of the Instituto Biologico, São Paulo, Brazil, kindly supplied two large collections of *Conchaspis lepagei* HEMPEL.

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AN EXPERIMENTAL AND DESCRIPTIVE STUDY OF CHROMOSOME ELIMINATION IN MIASTOR SPEC. (CECIDOMYIDAE; DIPTERA)*

By

R. Bruce Nicklas

With 48 Figures in the Text

(Eingegangen am 22. Januar 1958)

A. Introduction

Chromosome elimination as a normal feature in the differentiation of somatic cells poses many problems of cytological and genetical interest. Among animals such exceptional chromosome behavior is known to occur in certain families of Diptera: the *Chironomidae*, the *Sciaridae*, and the *Cecidomyidae*. Some peculiarities in the embryology of the last named group were already noted by Metschnikoff in 1866, but it remained for Kahle in 1908 to give the first information on chromosomal behavior in one of the species. Kahle reported that in *Miastor* a considerable amount of chromosomal material is eliminated in certain early cleavages, and that it is this elimination that differentiates the future somatic nuclei from the unaffected germ cell nuclei.

The previous work on the general embryology and lifecycle of *Miastor* has been reviewed in detail by White (1946) and Hagan (1951). It is sufficient to say here that in this report we shall be concerned with female embryos that develop paedogenetically by apomictic parthenogenesis, in which the egg undergoes only an equational maturation division. The first two divisions of the cleavage nucleus are perfectly normal, but during the third cleavage in most embryos there is the first indication of a loss of chromosome material. The amount of this material is variable but none is ever lost from the most posterior nucleus. The division of this posterior nucleus at Cleavage III gives rise to one nucleus that is segregated by cytokinesis in the pole plasm and represents the ancestor of all the germ cells. Except in the primordial germ cell, a further loss of chromatin occurs in the fourth cleavage of *Miastor*, and this ends the elimination process. These early nuclear divisions, called "cleavages" for convenience, are *not* followed by cytokinesis except for the divisions of the primordial germ cell.

* From a dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, in the Faculty of Pure Science, Columbia University.

The problems presented by chromosome elimination in the Cecidomyidae are as follows:

1. The pioneering work of Kahle represents a correct analysis of the general embryology of *Miastor*, as given above. However, he believed that the loss of chromatin involved only parts, not whole chromosomes. Many years later Huettner (1934) and Reitberger (on *Oligarces*, 1934) came to the conclusion that entire chromosomes are eliminated, and in this they were undoubtedly correct. Huettner (1934) also suggested that the somatic chromosomes represent a diploid set segregated from a polyploid germ-line chromosome complement, and most subsequent workers on various cecidomyids have agreed (Reitberger 1939; Kraczkiewicz 1935, 1936; White 1946, 1947a). White (1947b, 1950) came to doubt this conclusion, but hitherto no decisive evidence on this point has been available.

2. The cytological features that characterize the process of elimination in *Miastor* have not been adequately described. In *Oligarces* (Reitberger 1939) this has been done, but unfortunately ambiguity still exists as to exactly what the mitotic failure is, morphologically. It is manifest that an exact knowledge of this is prerequisite to an understanding of the cell mechanics that underlie elimination.

3. Recent advances in cytochemical techniques offer an opportunity to determine whether the exceptional cytological conditions are correlated with detectable chemical changes.

4. In the cecidomyids chromosome elimination is limited to a certain number of chromosomes, to certain nuclei, and to a particular time in the life-cycle. An understanding of how these specificities are determined is the core of the problem as a whole. As a first step, an inquiry may be made into the role played be extra-chromosomal factors as opposed to intrinsic properties of the chromosomes themselves.

It is a great pleasure to acknowledge the kind guidance and sponsorship of Professor Franz Schrader. I am also deeply indebted to Professor Arthur W. Pollister and to Dr. Sally Hughes-Schrader.

B. Material and methods

The larvae used in this study were a clone derived from a single parthenogenetic female — one of hundreds collected near Alpine, New Jersey in the decaying bast of white oak logs. They represent a species of *Miastor* that has not been previously studied cytologically. While the generic attribution is certain, identification of the species must await the production of adult forms.

The basic requirement for any detailed study of cecidomyid cytology is an efficient culture method which can furnish an adequate supply of all required stages. The malt broth method devised by Ulrich (1936) was tried with a variety of molds as the food source. These preliminary experiments failed, and only the mold that was kindly sent to me by Mr. Ehrenfried Nikolei of the Eidgenössische

Technische Hochschule in Zürich permitted the maintainance of flourishing cultures. I am grateful to him as well as to Professor ULRICH for their generosity and helpfulness. The temperature used was 29° C, at which a single generation lasts about one week. About two days elapse between the time the daughter larvae leave the mother's skin and the time when they contain embryos in the early cleavage stages. The stage of the embryos in individual larvae can be determined to within about two cleavages by examination with a dissecting microscope. Larvae from ten different generations were used in this study. A total of more than thirty generations have now been reared with virtually no unnatural deaths.

For cytological examination embryos of the desired stages were liberated from the mother, larvae with dissecting needles, collected in Beadle and Ephrussi insect Ringer's and fixed in Sanfelice or very weak Flemming. Both fixatives, at their best, give fine preservation of chromosomes, but the Sanfelice is less variable. Whole larvae with their contained embryos were punctured and fixed in 1:3 acetic-alcohol for use in the cytochemical studies. Often chromosome preservation after such treatment is very fine, and then the preparations were used cytologically as well.

Feulgen and iron hematoxylin staining of sectioned material was used for cytological study.

Hitherto, good squash procedures for such embryos have not been available. The following one will give fine results for chromosome number and morphology. The embryos are dissected out into distilled water (MAKINO and NISHIMURA 1952). After about twenty minutes, they are flooded with a modified Kahle fixative: 30 parts distilled water: 12 parts 95% alcohol: 6 parts glacial acetic acid: 2 parts of 37% formaldehyde. After about fifteen minutes, the embryos are transferred to a slide and squashed lightly with an albuminized coverslip. The coverslip is removed, and a conventional Feulgen stain follows. In favorable metaphases from such preparations, chromosome number and kinetochore position are very clear. Chromosome lenghts and kinetochore positions were measured directly with an ocular micrometer.

If the distilled water pretreatment is omitted and conventional Kahle fixation is used in the above procedure, slightly flattened whole mounts rather than squashes are obtained. Such preparations have been used extensively in the study of centrifugation effects. Even details of chromosome behavior and size are very well preserved, as a comparison with sectioned material will attest (compare Figs. 42 and 48 with Figs. 21 to 25).

The cytochemistry of these embryos was studied both on acetic-alcohol and on alcohol frozen-substituted (by the methods of WOODS and POLLISTER, 1955) material. The former was used in the quantitative study of Feulgen stained chromosomes. Both types of fixation were used for the qualitative studies of RNA, protein and polysaccharides.

RNA (ribose nucleic acid) basophilia was studied with the pH 4.0 Azure B method of FLAX and HIMES (1952). A control was always run after predigestion with proteasefree RNase (KAUFMANN et al. 1951).

The periodic acid Schiff reaction was used as suggested by HOTCHKISS (1948) for the detection of 1,2 glycol linkages. Predigestion with saliva (1 hour at 40° C) was used to show the contribution of glycogen to this reaction.

Protein tyrosine was visualized according to the POLLISTER and MIRSKY (1946) modification of Millon's reaction.

Acidophilia of basic proteins was studied with the ALFERT and GESCHWIND (1953) alkaline fast green reaction, using the suggested formaldehyde fixation. Controls without the trichloracetic acid (TCA) treatment prior to staining were also run, as suggested to me by Dr. ARLINE DEITCH of Columbia University (private communication). RAase digestion (KAUFMANN et al. 1951) instead of the usual TCA treatment was also used.

For the cytophotometric studies of DNA (desoxyribose nucleic acid) the Feulgen reaction according to Leuchtenberger (1958) was used. The use of Feulgen cytophotometry to determine the relative amount of DNA in nuclei is well established (Swift 1953, Leuchtenberger 1954, Pollister and Ornstein 1955). The slides were hydrolyzed 10 minutes at 60° C. Unhydrolyzed controls were always run, and were uniformly negative. All Feulgen-positive material is digestable by incubation in Worthington DNase by the method of Leuchtenberger (1958).

The division figures to be measured were selected with regard only to their being uncut, and were mapped for later measurement of their DNA. The refractive index of the nuclei was matched to that of the surrounding medium by using the appropriate Cargille Laboratories oil, as determined by phase microscopy.

The amount of DNA in various mitotic stages was measured by the two wavelenght method (Ornstein 1952, Patau 1952). The apparatus used has been described by Ansley (1956). To this was added a Perkin-Elmer model 83 monochrometer and tungsten ribbon filament light source. The wavelengths used to make the measurements were chosen from extinction vs. wavelenght curves from homogeneous somatic blastulae nuclei so that the extinction at the second wavelength is twice that found at the first wavelength (Patau 1952). The second wavelength was chosen near the absorption peak in order to obtain maximal extinctions at the first wavelength. This was necessary because otherwise extinctions at the first wavelength occasionally would fall below 0.10 in the division figures measured. The wavelengths used were 509 and 565 millimicrons, with a nominal band pass of about 2 millimicrons at each wavelength. The instrument was checked before use with the tests of Ornstein (Pollister and Ornstein 1955). Phototube response was linear from the smallest to the largest plug sizes used. Fixed plugs were used giving greater accuracy and reproducibility than a movable diaphragm (Patau 1952). Repeat determinations on 11 nuclei were reproducible to within 3%. The values are expressed in arbitrary amounts calculated by Patau's method.

The centrifugation experiments were carried out on larvae with embryos whose age was roughly established with a dissecting microscope. Preliminary study showed that after the maturation division, each early cleavage last about one hour from one metaphase to the next, in both normal and centrifuged material. Thus the age of any individual embryo can be approximately determined by knowing the elapsed time between centrifugation and fixation, and the stage at fixation.

The larvae were centrifuged 25 minutes at 2400×g in a small Adams CT-1004 centrifuge. After various time intervals from 0—20 hours, the embryos were dissected out, fixed in Kahle, and prepared as Feulgen whole mounts as described above. A total of 16 different runs was made, and the results pooled. One batch was fixed 20 minutes after centrifugation in Flemming-without-acetic, and unstained whole mounts were prepared. These were examined with a phase contrast microscope to determine the distribution of both nuclei and cytoplasm.

C. Results

I. Cytological observations

1. Chromosome number and morphology

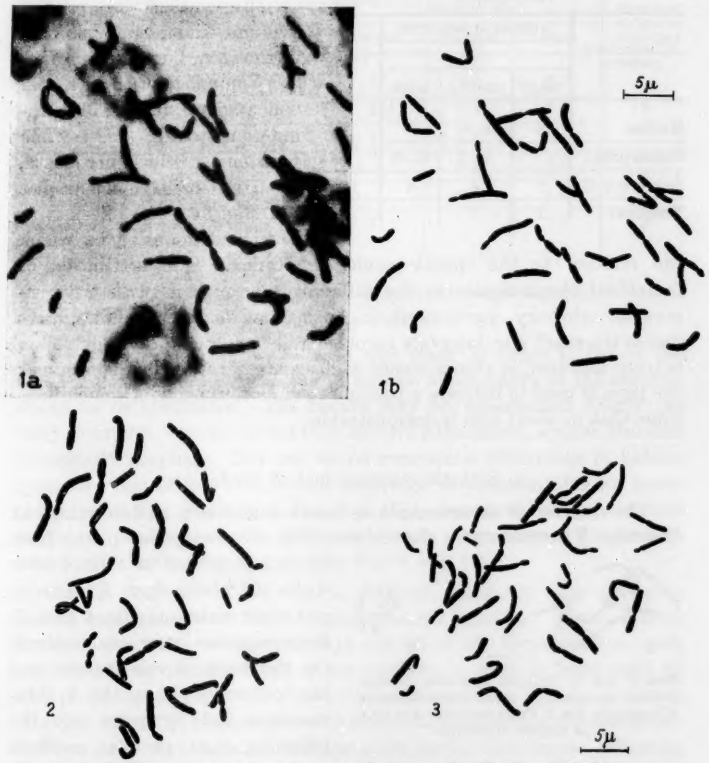
a) The germ-line chromosomes

The germ-line number of chromosomes varies between 38 and 40 among different individuals. Table 1 shows the distribution found in twelve perfectly countable metaphases from squash preparations of

early cleavage nuclei. Fig. 1, 2 and 3 show examples of 38, 39 and 40 chromosome plates, respectively. The deviations in number appear to be genuine and not due to counting mistakes or loss of chromosomes in squashing. The latter is ruled out by the presence in the preparation of the whole embryo.

Table 1. *Germ-line chromosome number*

Number of chromosomes	38	39	40
Number of plates . . .	2	8	2



Figs. 1—3. Feulgen squashes. Germ-line metaphases showing 38, 39 and 40 chromosomes, respectively

These differences in number most probably arise by non-disjunction, particularly of the smallest chromosomes with median kinetochores. A count of all plates in any one embryo, as a partial test of this hypothesis, is usually impossible, but could be made in one Cleavage II

embryo. Here the anterior and posterior metaphases showed 37 and 39 chromosomes respectively. This is particularly instructive since the more posterior nucleus will give rise to the primordial germ cell nucleus, The case thus demonstrates how non-disjunction (?) could raise the germ-line number back to the mode of 39 in the progeny of an embryo that originally had 38 chromosomes.

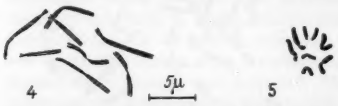
Table 2. *Morphology of the germ-line chromosomes*

Kinetochore position	Number of each type			Total
	Size			
	small	medium	large	
Median . .	3	4—6		21
Submedian .		5—7	6—8	
Subterminal	2	4	4	10
Terminal .	1	7		8

Observations on relative size and kinetochore position in the germ line chromosomes are summarized in Table 2, which gives data for 39-chromosome plates only. The position of the kinetochore, clearly identifiable in squashes (cf. Fig. 1a), was checked in sectioned material in which the relation to the spindle could be verified. The attribution of individual chromosomes to the different subgroups is necessarily somewhat arbitrary, particularly in the median and submedian c, lasses due to the small size intervals involved. Wheter or not the kinetochore is truly terminal, in chromosomes so designated, cannot be determined; the term is used to indicate a position so close to the end of the chromosome that no short arm is demonstrable.

b) Somatic chromosomes of the female

The number of chromosomes in female nuclei is 8, as determined at Cleavage V immediately after elimination (57 metaphase plates from 12 embryos), and in cells of later stages (27 plates from 8 blastulae and older embryos) (Fig. 4 and 5). No exceptions were encountered. On the basis of relative size and kinetochore position, the 8 chromosomes fall by pairs into the following four groups: medium size, submedian kinetochore; large — submedian; medium — subterminal; and large — subterminal.



Figs. 4 and 5. Feulgen squashes. Female somatic metaphases. Fig. 4. From Cleavage VII embryo. Fig. 5. From embryo at the time of somite formation

A comparison of the morphology of germ-line and somatic chromosomes shows that those chromosomes which are retained in the soma fall in the medium and large size categories — the small chromosomes are not represented. The latter may be found, as expected, among the eli-

minated chromosomes in squashes of Cleavage V embryos. These conclusions are established by measurements of the length of individual chromosomes, and these data are summarized as ratios in Table 3. In each type — germ-line, somatic, and eliminated chromosomes — the chromosomes used as the basis for the ratio are indicated by a superscript.

Table 3. *Relative chromosomal size (see text)*

Type of figure	Kinetochore position	Relative size			Number of figures measured
		small	medium	large	
Germ-line metaphase	median—submedian	1[1]	2.5	3.0	9
	subterminal	1.6	2.5	3.0	
	terminal	1	1.8		
Somatic metaphase	Submedian		2.5[1]	3.0	8
	subterminal		2.0	3.0	
Eliminated chromosomes	median—submedian	1[1]		3.0	9

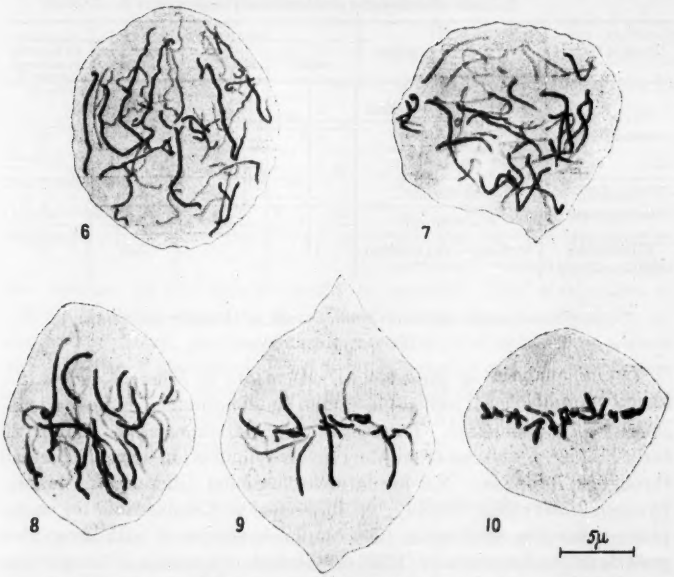
2. Chromosome behavior from prophase through metaphase in young embryos

Careful analysis of these stages was made in order to detect the earliest morphological indication of mitotic abnormality in the chromosomes to be eliminated. The results may be summarized briefly: all early cleavages, whether or not they involve elimination, appear identical throughout prophase. Nor are interchromosomal differences in heteropycnosis observable. Finally, no difference is demonstrable at metaphase, when the elimination Cleavage IV is compared with those that precede it. As REITBERGER (1939) established, congression of the chromosomes is perfectly normal (compare Fig. 9 and 10).

Certain features of cytological interest, although not obviously related to elimination, deserve parenthetical mention. Thus, a nonrandom orientation of most, though not all of the chromosomes, such that their long axes lie more or less parallel, is evident from early to mid prophase (Fig. 6). This orientation disappears in late prophase (Fig. 7). Secondly, premetaphase to late metaphase stages are characterized by a series of changes in the shape of the nuclear membrane, reflecting the changing form of the spindle within it, and probably indicative of varying activity of the spindle fibers (Fig. 8—10). These changes are not associated with the dissolution of the nuclear membrane, since this does not occur until anaphase (Fig. 13, 21 and 24).

The clean separation of daughter chromatids at metaphase should be emphasized. The split visible in late pre-elimination metaphase in

Fig. 10, also seen in elimination metaphase, is significant. If stickiness or protracted cohesion were a factor in elimination, in might be expected that this split would not be seen. Furthermore, experimental evidence on the relation of sister chromatids at late metaphase of the elimination Cleavage IV was obtained from squashes (Fig. 11). Sister chromatids are usually separate throughout their lenght, and invariable so at their



Figs. 6—10. Figs. 6—9. Flemming; iron hematoxylin. Fig. 10. Acetic-alcohol; Feulgen. Fig. 6. Cleavage II mid-prophase. Fig. 7. Cleavage II late prophase. Fig. 8. Cleavage II premetaphase. Fig. 9. Cleavage IV early metaphase. Fig. 10. Maturation division late metaphase

ends. This was checked in 20 plates from 6 embryos. The degree of separation varies in different plates but, significantly, is uniform within any one plate. Precisely similar conditions obtain in non-elimination cleavages.

3. Anaphase to telophase in non-elimination cleavages

Non-elimination anaphases are shown in Fig. 12 and 13. Synchronization of chromatid movement is good but not perfect. Before separation, the ends of daughter chromatids come into contact. This is seen in the longer chromatids in Fig. 12. It is difficult to determine, in most cases, whether such ends are intertwined or merely touching

each other, and both alternatives may occur in different chromosomes in the same division figure. For example, simple touching is indicated in two chromosomes at the right side of Fig. 12, while in one chromosome near the left side, intertwining of ends may have occurred. In either case separation is clean (Fig. 13).

The very early telophase of Fig. 14 shows the great elongation just undergone by the spindle. The parallel arrangement of chromosome arms in telophase is here clearly apparent.

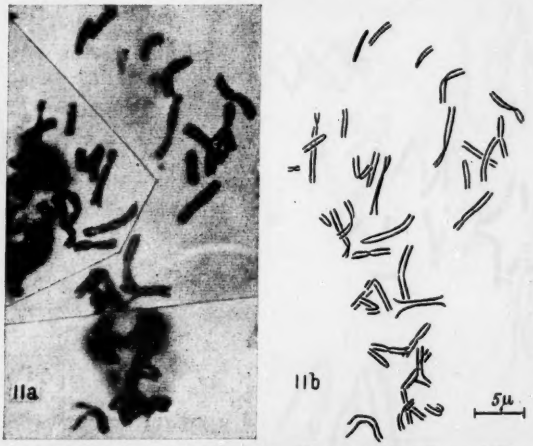


Fig. 11 a and b. Feulgen squash. Daughter chromatid separation in a Cleavage IV metaphase. The photograph is a montage from two focal levels

Further details of the ordinary anaphase behavior are observable in post-elimination somatic mitoses. The ends of daughter chromatids are completely separate in very early anaphase (Fig. 15), as they have been from late metaphase. As anaphase proceeds, however, these ends are drawn into contact (Fig. 16). This behavior is constant and typical of anaphase in this insect: it has been seen without exception in a hundred or more division figures at this stage. Here, in contrast to Fig. 12, one can usually be sure that the ends are merely touching, and are not intertwined. The later stages (Fig. 17 and 18) show the continued strong tendency to synchrony in chromosome movement.

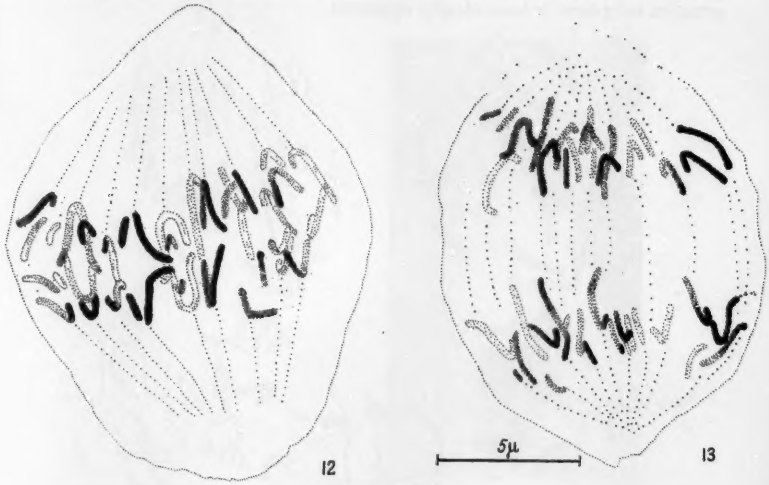
4. Anaphase to telophase in elimination cleavages

The preceeding sections have shown that elimination and non-elimination cleavages appear identical through prophase, premetaphase and metaphase and have presented the anaphase behavior of the

chromosomes in non-elimination cleavages. The present section gives cytological data on anaphase and telophase in the elimination cleavages (III and IV).

a) The nature of the material eliminated at Cleavage III

Before considering the differential behavior of chromosomes in elimination anaphases it is necessary to fill in one gap in the record:

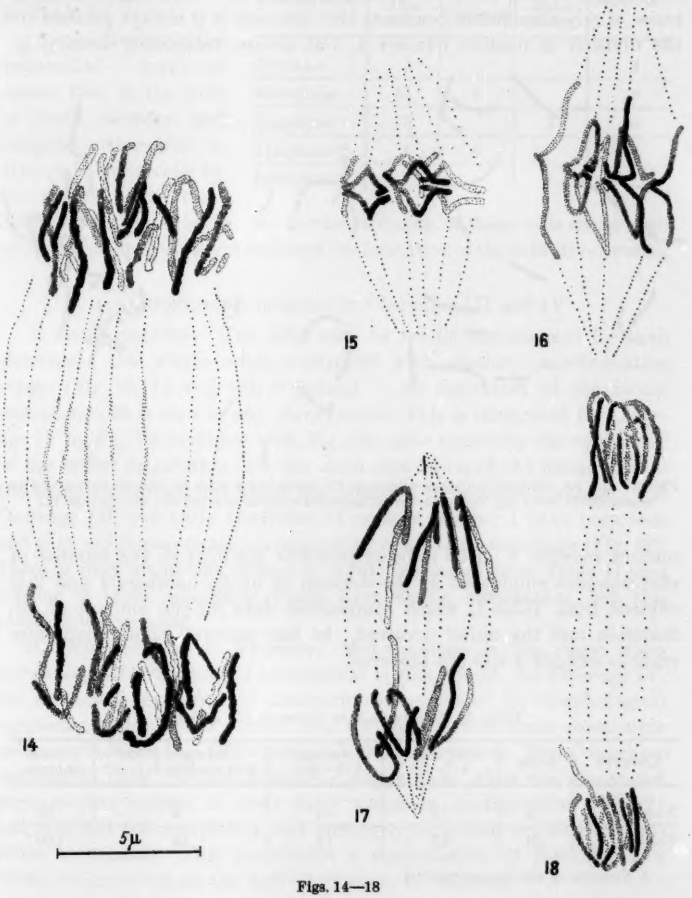


Figs. 12 and 13

Figs. 12—18. Non-elimination anaphase to telophase. Not all of the chromosome are figured. Figs. 12 and 13. Acetic-alcohol; Feulgen. Fig. 14. Flemming; iron hematoxylin. Figs. 15—18. Sanfelice; Feulgen. Fig. 12. Cleavage II early anaphase. Fig. 13. Cleavage II mid-anaphase. Fig. 14. Maturation division early telophase. Figs. 15—18. Mid-blastula somatic division figures. Fig. 15. Very early anaphase. Fig. 16. Early anaphase. Fig. 17. Mid-anaphase. Fig. 18. Late anaphase

while it has previously been demonstrated that whole chromosomes are eliminated at Cleavage IV in *Miastor* (Kraczkiewicz 1935, 1936), the nature of the material eliminated at Cleavage III has never been established. The question is settled by examination of squash preparations of Cleavage IV metaphases derived from nuclei which had undergone elimination at Cleavage III. Four countable plates of this type were found, and two of these are shown in Fig. 19 and 20. These figures make it obvious that whole chromosomes have been lost. Additional information can now be adduced, based on the data on chromosome morphology presented in section 1a. In the two completely analyzable plates (Fig. 19 and 20) it is found that the size and kinetochore positions of the chromosomes surviving to Cleavage IV metaphase fit without

exception into the groups in the germ-line described earlier (Table 2). Therefore there has been no loss of parts of the chromosomes that remain; whole chromosomes and *only* whole chromosomes are lost here, as in Cleavage IV.

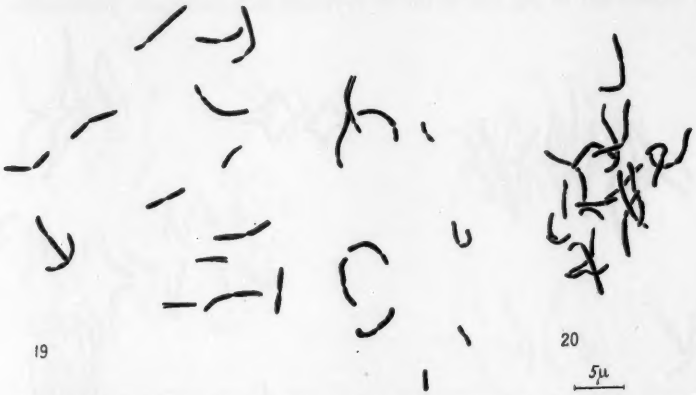


Figs. 14—18

b) The general pattern of nuclear behavior in anaphase to telophase of Cleavages III and IV

The general features of Cleavages III and IV were established by KAHLE, but it is necessary to add here the range of variation encountered.

At Cleavage III metaphase the four cleavage nuclei are scattered from the anterior to close to the posterior end of the embryo. Following KAHLE's practice, I shall designate them and their pairs of daughter nuclei by the numbers 1 to 4, starting at the anterior end. As KAHLE knew, if any elimination occurs at this cleavage it is always greatest in the division of nucleus number 1, but always completely lacking in



Figs. 19 and 20. Feulgen squashes. Cleavage IV metaphases with 25 and 19 chromosomes respectively, showing loss of chromosomes from nucleus number one at Cleavage III

nucleus number 4. This anterior-posterior gradient in the number of chromosomes eliminated in the division of nuclei numbers 1 and 2 is evident from Table 4, which summarizes data on the amount of elimination and the nuclei involved. In this material elimination from nucleus number 3 was not observed.

Table 4. *Elimination in Cleavage III embryos*

Embryos	None	Nucleus 1: 1—5[1]	Nucleus 1: 6—20[1]	Nucleus 1: 6—20[1] and nucleus 2: 1—5[1]	Total embryos
Number .	8	12	4	4	28
Percent .	29	43	14	14	100

[1] Number of chromosomes lost.

Cleavage IV anaphase, in contrast to Cleavage III, has a very precise result: all fourteen daughter nuclei formed contain only 8 chromosomes (see 1b, above), and these are the future somatic nuclei. As KAHLE stated, the primordial germ-cell does not divide at this time. The range,

in my material, of its mitotic stage for various times during Cleavage IV in 36 embryos is given in Table 5.

Table 5. *Behavior of the primordial germ-cell nucleus during Cleavage IV*

Stage of future somatic nuclei	Stage of the germ-cell nucleus			Total no. of embryos
	Interphase	Prophase	Metaphase	
Prophase . .	3			3
Metaphase .	15	1		16
Anaphase .	9		1	10
Telophase .	1	5		6
Interphase .			1	1

Actual division of the primordial germ-cell occurs first at the fifth or sixth cleavage and irregularly thereafter to give eight germ-cells by the middle of the blastula stage in all embryos. No further divisions of these cells ensue until after migration of the germ-cells and the formation of the definitive ovaries.

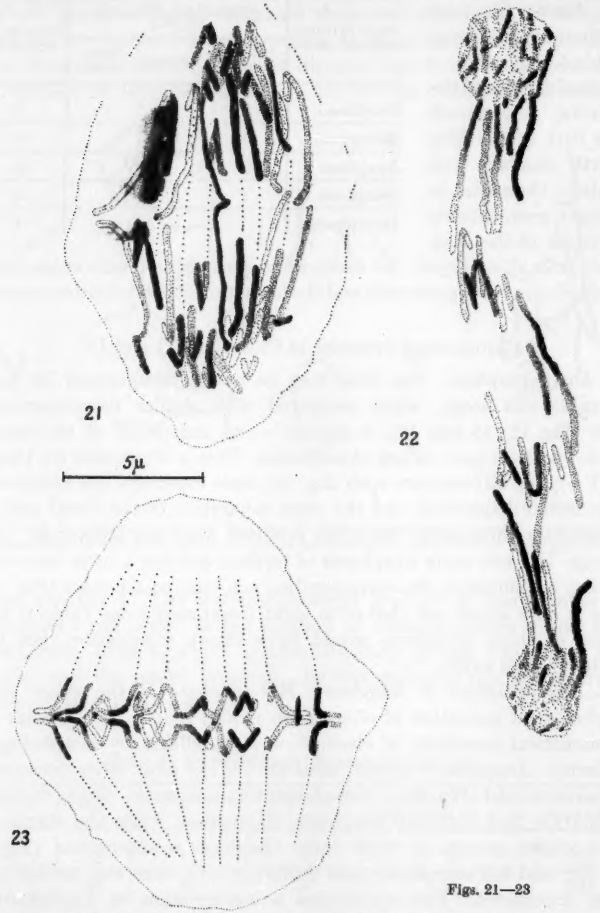
c) Chromosome behavior in Cleavages III and IV

i. Early anaphase. The data may be briefly summarized for both cleavages: this stage, when compared with similar non-elimination stages (Fig. 12, 15 and 16), is normal — no indication of the future mitotic failure is seen in any chromosome. This is illustrated for Cleavage IV by Fig. 23 (compare with Fig. 15); note especially the synchrony of the initial disjunction and the clean separation of the distal ends of the daughter chromatids which has presisted from late metaphase. For Cleavage III, two early anaphases of nucleus number 1 have been seen and they are similar to the corresponding non-elimination stage (Fig. 12). There is only about one chance in eight (computed from Table 4) that neither of these anaphases would have shown elimination, but this possibility does exist.

ii. Mid-anaphase to telophase. Mid-anaphase is the stage when morphological indication of elimination is first found. In Cleavage IV, the numerical regularity of elimination is paralleled by morphological regularity. Anaphase is normal until the ends of most sister chromatids are in contact (cf. Fig. 16 — non-elimination anaphase). Eight daughter chromatids then continue anaphasic disjunction, while the eliminated chromosomes remain in their early anaphase configuration (Fig. 24 and 25), and fail completely and uniformly to evince any further anaphase movement. This constitutes a confirmation of REITBERGER's (1939) observations on the first elimination in *Oligarces*.

Cleavage III elimination, however, presents a rather different picture (Fig. 21 and 22). Almost all the daughter chromatids separate from each other and move some distance toward opposite poles, but there is great interchromosomal variation in the amount of this movement. There is no evidence that chromatids once separated later re-establish

the terminal connection, as occurs in the first elimination in *Oligarces* (REITBERGER 1939). The study of these and fifteen similar anaphases



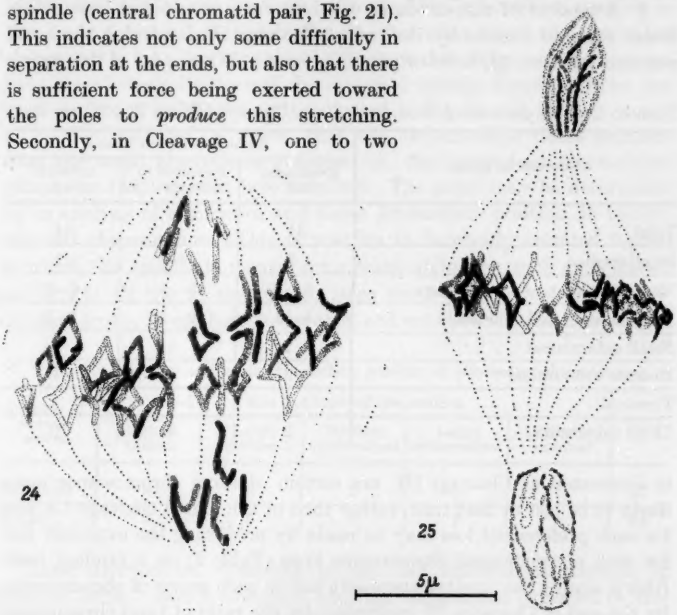
Figs. 21—23

Figs. 21—25. Elimination anaphase to telophase. Not all of the chromosomes are figured. Fig. 21. Carnoy; Feulgen. Figs. 22—25. Sanfelice; Feulgen. Figs. 21 and 22. Cleavage III; nucleus number one. Fig. 21. Mid-anaphase. Fig. 22. Telophase. Figs. 23—25. Cleavage IV. Fig. 23. Very early anaphase. Fig. 24. Mid-anaphase. Fig. 25. Telophase

and early telophases also demonstrates that there in *no* correlation between the amount of anaphase movement and the following factors:

size of the chromosome, its kinetochore position, and the chromosome's position on the metaphase plate.

Exceptions to the general behavior of eliminated chromosomes occur in both elimination cleavages. These exceptions bear on the immediate cause of elimination and are as follows: firstly, in Cleavage III an occasional chromatid pair will fail to separate and will be stretched on the spindle (central chromatid pair, Fig. 21). This indicates not only some difficulty in separation at the ends, but also that there is sufficient force being exerted toward the poles to *produce* this stretching. Secondly, in Cleavage IV, one to two



Figs. 24 and 25

chromatid pairs in the eliminated group remain separate at the ends throughout anaphase, and yet fail to move at anaphase. This is shown in chromatids just to the right of center in Fig. 24 and 25. One or two such pairs of daughter chromatids were found in every one of the 21 division figures studied, which cover the entire period of this stage.

An interesting phenomenon — whose precise significance remains unknown — is disclosed by elimination anaphase both here (Cleavage IV) and in the first elimination in *Oligarces* (Reitberger 1939). When the non-eliminated chromosomes move in anaphase, they leave a gap in the plate of eliminated chromosomes just off the central axis of the plate (Fig. 24 and 25). This means that the position of the chromosomes

that behave normally is determined at least as far back as the time of congression to the elimination metaphase plate.

iii. Cytological analysis of questions posed by anaphase in Cleavages III and IV. Two questions concerning elimination in *Miastor*, which require a knowledge of chromosomes morphology for their solution, can now be considered.

1. A total of 31 chromosomes will have been eliminated from every future somatic nucleus by the end of Cleavage IV (assuming the most common number, 39, to be originally present). When part of this group

Table 6. *Test for preferential chromosome loss at Cleavage III* (for Fig. 26; see text)

Chromosome group	Number of chromosomes		
	Eventually lost	Expected to be lost	Actually lost
Total	31	—	14
Small median	3	1—2	1
Medium median and submedian	8—10	3—5	4—6
Large median and submedian	4—6	2—3	1—3
Small subterminal	2	1	0
Medium subterminal	2	1	1
Terminal	8	3—4	5
Large subterminal	2	1	0

is eliminated at Cleavage III, are certain of these chromosomes more likely to be lost at that time, rather than at the fourth cleavage ? A test for such preferential loss may be made by predicting the expected loss for each morphological chromosome type (Table 2) on a random basis [this is simply the number *eventually* lost in each group of chromosomes by the end of Cleavage IV multiplied by the ratio of total chromosome loss in any one particular Cleavage III anaphase to the total number (31) eventually lost]. As an example, such a compilation is made in Table 6 for the chromosomes shown in Fig. 19. The number actually lost in each group is computed by substracting the number *present* in Fig. 19 from the number original present (Table 2).

When the chromosomes in Fig. 20 are analyzed in the same way, the results are essentially the same, except that instead of the terminal group being higher, and the subterminal group being lower than expected, the converse is true. Therefore, no one chromosome group appears much more likely to lose chromosomes at Cleavage III than any other group. This probably, but not necessarily, applies to individual chromosomes as well. Obviously this is far too small a sample to detect any except rather gross differences in susceptibility to loss.

2. Previous workers have not considered whether or not the orientation of chromosomes to the poles is normal in chromosome elimination. From REITBERGER's (1939) and WHITE's (1950) illustrations of elimination in *Oligarces* and *Monarthropalpus* respectively, it is clear that the orientation of the normally behaving chromosomes is orthodox — i.e. the kinetochores are orientated toward the poles. This is also true in *Miastor*, as is shown by a comparison of the kinetochore position in Fig. 4 and 5 with orientation in Fig. 24. But what about the eliminated chromosomes ? As REITBERGER (1939) showed in *Oligarces*, their apexes have morphologically normal chromosomal spindle fibers, and this has been confirmed in *Miastor*, as is indicated in Fig. 24 and 25. It remains to be demonstrated, however, that the chromosomal fibers emanate from the usual kinetochore position, i.e., that secondary kinetochores (RHOADES 1952) are not here involved. The point may be determined by an analysis of arm ratios and hence kinetochore position, in the eliminated chromosomes. This is possible in favorably oriented figures in which the spindle is viewed some what obliquely, such as is shown in Fig. 24. Of the 25 medium to large sized chromosomes in the eliminated group, 24 are here analyzable, and are shown in the illustration.

Table 7. *Expected and actual kinetochore position in eliminated chromosomes*

Number of chromosomes	Size and kinetochore position					Total
	Medium median	Large median	Medium subterminal	Large subterminal	Medium terminal	
Expected	9	5	2	2	7	25
Actual . .	9	5	2	2	6	24

Their distribution, according to size and kinetochore position, is given in Table 7, and is compared with the distribution expected on the basis of the relative frequency of these morphological types in the germline chromosomes (Table 2) minus the somatic chromosomes. The good agreement between the expected and actual distribution shown in Table 7 was replicated in two other plates analyzed. It thus seems clear that the chromatids of the eliminated chromosomes are orientated in the orthodox manner, i.e., with their primary kinetochores toward opposite poles.

II. Cytochemical observations

1. Feulgen cytophotometry of DNA

The data presented are two wavelenght measurements of the relative amounts of Feulgen dye in metaphases of various stages. Some individual anaphase groups are also included (multiplied by a factor of two, of course). Interphases and prophases cannot be reliably measured by

any method because extinctions are too low. The mean values and their standard errors are given in Table 8, and the data are presented in histogram form in Fig. 26.

Table 8. *Relative amounts of DNA*

Stage and type of nuclei	Number of nuclei measured	Mean amount of DNA	Standard error of the mean
Germ-line before normal division	18	6.91	0.16
Germ-line before Cleavage III elimination .	16	6.86	0.24
Germ-line before Cleavage IV elimination .	14	7.25	0.13
Germ-line: Grand mean	48	7.00	0.11
Somatic: Early cleavage	20	1.85	0.04
Elimination clumps: Cleavage VI.	8	5.06	0.63

"Germ-line nuclei" as used in this table simply designates nuclei with the germ-line number of chromosomes, and does not imply their fate. All the values in the "before Cleavage III elimination" group are metaphases of nuclei numbers one or two. The "before Cleavage IV elimination" group includes metaphases and anaphases that did not lose chromosomes at Cleavage III, but would be certain to do so at Cleavage IV. Four of the values in the last group are from measurements on whole elimination anaphases. The somatic DNA values are from measurements on early cleavage metaphases.

These data make it clear that the amount of DNA synthesized is normal in the chromosomes to be eliminated, since significant differences between the first three groups do not exist. If no DNA were synthesized in the chromosomes to be eliminated, the DNA value for cleavage four metaphase should be depressed by 37% $\left[\frac{6{,}91-1{,}85}{6{,}91\times 2}\times 100\right]$. This would be easily detectable if present. It is easily calculated that somatic nuclei contain 74% less DNA than the germ-line nuclei at an equivalent synthesis stage.

The masses of eliminated chromosomes rapidly become pycnotic, and are difficult to identify in later cleavages. However, it was possible to measure the amount of DNA in 8 such masses from three Cleavage VI embryos. In these embryos there had been no elimination at Cleavage III, and all the Cleavage IV masses could be identified. It was also necessary to be certain that all 14 somatic nuclei are present, since degenerating somatic nuclei (Reitberger 1939) might fuse with the elimination masses. These data are given in Table 8 and Fig. 26.

The mean for these values (5.06) is not significantly different from the value expected just after Cleavage IV (5.15 = 7.00—1.85). This, and the histogram, make it likely that loss of Feulgen-DNA has already begun, and make it certain that no significant of DNA synthesis has taken place in the eliminated chromosomes since Cleavage IV anaphase.

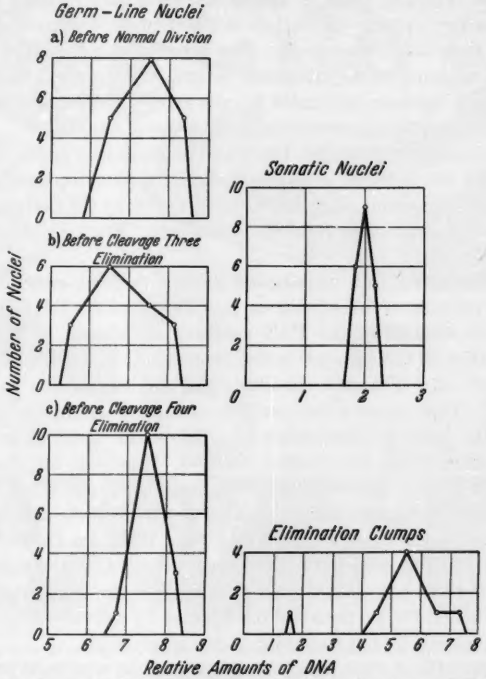


Fig. 26. The frequency distribution of DNA values in various types of division figures. In all histograms the ordinate represents the number of nuclei measured; the abscissa represents the relative amount of DNA

2. Distribution of RNA, protein, and polysaccharides

The most important result of these studies is that gross gradients of these substances in cytoplasmic areas of eggs or embryos do not exist.

The distribution of acide Azure B basophilia is shown in Figs. 27—29. The violet stain in the cytoplasm and nucleoli is completely digestible with protease-free RNase, and only the light green staining of chromatin is not. The ripening egg in Fig. 27 shows some staining in the cyto-

plasmic areas around the egg nucleus (touching the lower part of the nurse-cell chamber) and at the posterior end. In this particular embryo the staining is somewhat darker at the posterior end, but this is only rarely seen. The nurse-cell cytoplasm and nucleoli are intensely basophilic. The chromatin surrounding these nucleoli is faintly stained. By Cleavage IV (Fig. 28), there is almost no basophilia in the remains of the nurse-cell cytoplasm (to the left in the figure). The pycnotic nurse-cell nuclei stain an intense green. The primordial germ-cell lies at the right. The staining of its cytoplasm is little if any more intense than the cytoplasm between (indicated by the arrow) and around two nuclei that would have lost chromosomes in the ensuing anaphase.

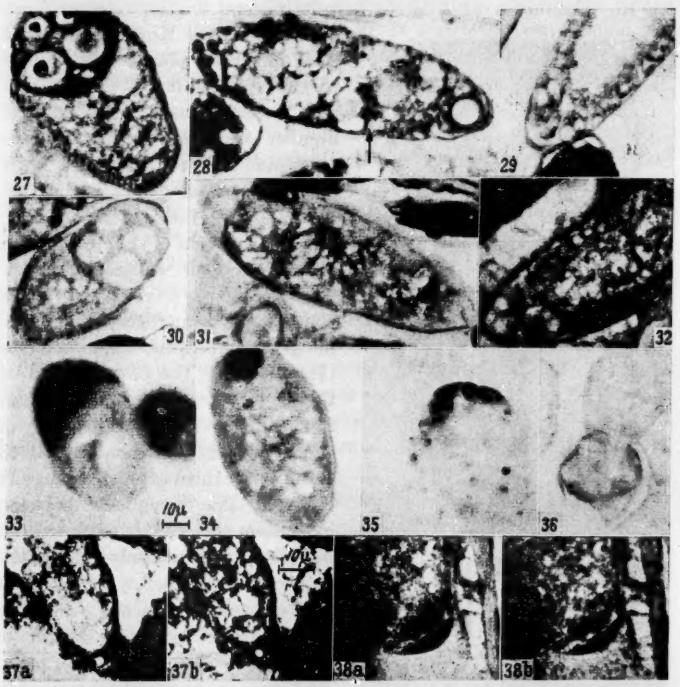
A mid-blastula is shown in Fig. 29. The germ-line nuclei are at the lower end of the embryo as illustrated. General cytoplasmic staining is seen, with no gradient. Nucleoli are just visible at the periphery of the somatic nuclei, particularly on the right side. They are first seen at about this stage.

The distribution and intensity of protein tyrosine as visualized by the Millon reaction is essentially as just described for RNA basophilia.

Embryos stained by the PAS reaction are shown in Fig. 30—32. The orientation of the embryos in the illustrations is essentially the same as in Fig. 27—29. The material illustrated was fixed with 3:1 alcohol acetic acid. This causes some variable artefacts, but in the illustrated embryos, the general distribution of stain is the same as in material handled by the freeze-substitution method. Gradients are absent from cytoplasmic areas. As contrasted with its appearance after RNA and protein stains the nurse-cell cytoplasm is at most no more intensely stained than the embryonic cytoplasm. Also, there is a gradual, general, increase in staining intensity from very young eggs through blastula stage. Nuclei are not stained. The intensity of the stain is reduced by about one-half if the sections are predigested by salivary amylase. This indicates that much of the staining is due to glycogen.

The Alfert-Geschwind basic fast green method was used to visualize nucleic-acid-bound basic proteins. It was immediately seen that quantitation of the nuclear staining would be impossible, since the cytoplasm also stains (Figs. 33 and 34). Such a result has also been obtained for some stages in *Tetrahymena* by Alfert and Goldstein (1955). This staining is not an artefact of faulty technique. No stain at all is found in controls not subjected to the TCA pretreatment to remove nucleic acid. Furthermore, some staining, limited to the cytoplasm, is found if protease-free RNase, rather than TCA is used as the pretreatment (Fig. 36). This indicates the presence of a histone-like protein bound to *cytoplasmic* RNA. Nucleoli, including the large ones of the nurse-cell nuclei, are never stained under any conditions. As shown in Fig. 33—35, the distribution of cytoplasmic staining is very similar to that for RNA.

The general cytochemistry of the polar granules has also been investigated. The freeze-substitution method was used for their preservation. These granules appear after such treatment as a more or less con-



Figs. 27—38. Peak transmission in millimicrons of the Farrand interference filters used in the photography is as follows: Azure B: 628; PAS: 557; Alkaline fast green: 628. Figs. 27 to 29. Azure B staining of a maturation prophase embryo, a Cleavage IV metaphase embryo, and a blastula, respectively. Figs. 30—32. PAS staining; stages as for 27—29. Figs. 33—35. Alkaline fast green staining after TCA of a maturation prophase embryo, a Cleavage II premetaphase embryo, and a Cleavage VI embryo, respectively. Fig. 36. Alkaline fast green staining after RNase of a maturation prophase embryo. Figs. 37 and 38. In both, *a* is a bright-field photomicrograph of the same area shown by phase contrast in *b*, where the polar granules are indicated by the arrow. Fig. 37. PAS. Fig. 38. Azure B

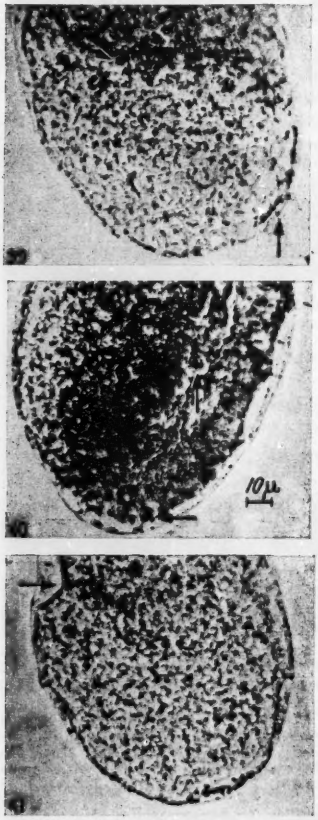
fluent mass, frequently broken away from the posterior wall of the embryo. They are indicated by arrows in Fig. 37 b and 38 b, which are phase photomicrographs of the same areas shown by bright-field in 37 a and 38 a.

PAS-positive materials are not present in these granules (Fig. 37 a and b). However, these granules do contain RNA, as indicated in Fig. 38 a and b. The Millon test for protein is also positive, but is not illustrated.

III. Observations on centrifuged embryos

1. General effects of centrifugation

Centrifugation between the maturation division and blastula stages for 15—30 minutes at 2400×g permits normal development in most *Miastor* embryos. However, embryos lying in certain planes with respect to the applied force, and at certain developmental stages, exibit a number of abnormalities. These abnormalities are associated with displacement of nuclei and cytoplasm toward the centrifugal pole, and displacement of yolk toward the centripetal pole. These primary effects can be seen in the phase contrast photomicrographs of Flemming-without-acetic whole mounts (Fig. 39—41). The posterior end of these embryos lies to the bottom in the illustrations. In Fig. 39, all the cytoplasm has been shifted to the anterior third of this Cleavage II embryo. The boundary between cytoplasm and yolk is very distinct and lies approximately along the axis of the left arrow. One of the two cleavage nuclei lies just below this arrow (most of it is out of focus). Its position is typical, for in such embryos the most posterior nucleus regularly lies at the boundary of cytoplasm and yolk. The converse is also true — if the cytoplasm has been moved posteriorally, then the most *anterior* nucleus indicates the extent of cytoplasmic movement. This result is useful in the interpretation of Feulgen-stained whole mounts, where the cytoplasm-yolk boundary cannot be seen.



Figs. 39—41. Centrifuged embryos. Flemming—without—acetic whole mounts; phase contrast microscopy. See text for details

A maturation telophase is shown in Fig. 40. One of the nuclei is indicated by the upper arrow. The cytoplasm lies to the right of this nucleus. Thus most of the cytoplasm has been thrown to the side

rather than anterior or posterior. The dark central area contains the other nucleus. This area is probably a mixture of cytoplasm and yolk. The latter occurs unmixed at the right in the illustration.

For comparison, a maturation prophase embryo showing no centrifugation effects is shown in Fig. 41. Yolk and cytoplasm remain unsegregated and no boundary is visible. The nurse-cell chamber lies above the arrow. Under these conditions, the prophase nucleus is not visible.

It is of considerable interest to note that the polar granules are never much displaced by this centrifugation procedure. They are indicated by the lower arrows in Fig. 39 and 40, and are clearly seen in Fig. 41. HOWLAND (1941) has found it equally difficult to shift the polar granules in *Drosophila* embryos.

Only certain particular patterns and amounts of nucleocytoplasmic displacements produce developmental abnormalities. A study of these (see below) makes it likely that embryos similar to those in Fig. 40 and 41 constitute a large proportion of the normally developing embryos.

Several groups of embryos have been followed to the stage of somite differentiation and beyond. All embryos persisting to such stages are normal in general aspect, and in regard to somatic chromosome complement and in the number and behavior of the germ-cells.

2. Effects upon Cleavage III

The effects on chromosome elimination at this cleavage are difficult to assess because of the normal variability of this division in control

Table 9. *Centrifugation effects on Cleavage III chromosome elimination* (in percent of a total of 18 embryos)

Amount of elimination and nuclei involved	Amount and direction of displacement				Total %
	Negligible	Slight anterior	Moderate anterior	Moderate posterior	
None		11	11	6	28
6—20[1]; nucleus 1 . .			6		6
6—20[1]; nuclei 1, 2 . .	22	11	22	6	61
6—20[1]; nuclei 1, 2 and 1—5[1]; nucleus 3 . .		6			6
Total	22	28	39	12	101

[1] Number of chromosomes lost.

material (Table 4). To facilitate discussion, embryos are classified according to the amount of displacement of their nuclei. The categories distinguished are; negligible, slight, moderate and large. The "negli-

gible" category, of course, refers to embryos which show essentially normal nuclear position. The last three categories are for embryos showing all the nuclei concentrated in about $^7/_8$, $^3/_4$, or $^1/_2$ respectively, of the embryo.

Table 9 shows the character of elimination at Cleavage III in percentages of a total of 18 embryos. All of these embryos were fixed between the anaphase of Cleavage III and the metaphase of Cleavage IV. Those in the negligible group were all centrifuged at about the maturation division. Of the remainder, seven had been centrifuged at about the maturation division, one at Cleavage I, and six at early Cleavage III.

Fig. 42. From Feulgen whole mount of a centrifuged Cleavage *III* embryo. Early telophase in nucleus number 1. Not all chromosomes are shown

If experimental embryos (Table 9) and control embryos (Table 4) are compared in regard to amount of elimination, the former are seen to show slightly more. However, two facts make it unlikely that this is anything other than the natural random variation inherent in chromosome elimination at this cleavage. First, the class showing negligible nuclear displacement also shows a relatively large amount of elimination. Furthermore, the "no elimination" class has about the same percent of embryos in both the experimentals and the controls, and all types of centrifugal displacements contribute to this class.

A division figure from a Cleavage III embryo showing negligible displacement of nuclei is shown in Fig. 42. This remarkable similarity to the Cleavage IV figures (Fig. 24 and 25) has also been seen in two other Cleavage III anaphases from different embryos. In all three examples, the number of eliminated chromosomes is relatively high.

3. Effects on chromosome elimination at Cleavage IV

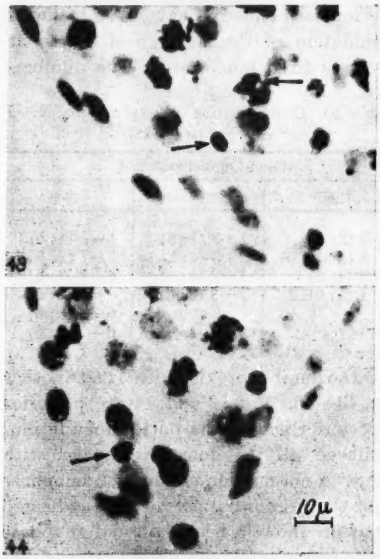
These effects are much more clear than if only Cleavage III is considered, because of the uniformity in control material at, and after, Cleavage IV. Many embryos with displaced nuclei show "extra non-eliminators" after Cleavage IV. These are nuclei that more or less

follow the behavior of the primordial germ-cell — they may or may not divide at Cleavage IV, but whenever they do divide there is little or no loss of chromosomes. These are considered "extra" because they are persent in addition to a more posterior nucleus which is equivalent to the primordial germ-cell nucleus. In Table 10 the number of extra non-eliminators is compared with the amount of nuclear displacement. Each number in the body of the table gives the number of "extras" for a particular embryo. Only those fourteen embryos have been included which permit cytological determination of the number of extras at Cleavage IV, even if the embryo was fixed much later. The numbers in parentheses give first the approximate cleavage at the time of centrifugation, and second the cleavage when the embryo was fixed ("mat." = maturation division; "bl." = blastula). The table strikingly illustrates one specificity of this effect — virtually without exception, the greater the displacement, the larger is the number of extras. This and another specificity are illustrated in Fig. 43—46. Fig. 43 and 44 are posterior portions of embryos, with the posterior end at the bottom of the illustration. Fig. 43 shows an embryo at the fourth cleavage with "moderate-anterior" displacement. A normal non-elimination telophase equivalent to the first division of the primordial germ-cell is indicated by the lower arrow. The upper arrow shows a nearly normal anaphase of an extra non-eliminator. Two metaphases are shown to the left above from this anaphase. Fig. 44 is of a sixth cleavage embryo in the "slight-posterior" group. The germ-line nucleus has already divided twice. The telophase of the upper two germ-line nuclei is indicated by the arrow, and lies perpendicularly to the axis of the other two germ-line nuclei which are in early interphase. Above this are two metaphases with about the germ-line number of chromosomes. Since approximately 24 normal somatic nuclei are present in this embryo, these two metaphases must be the result of the division, with little, if any, chromosome loss, of a single extra non-eliminator that was present at Cleavage IV. This is the last embryo in column one of Table 10. These embryos illustrate a second specificity of the effect of centrifugation — without exception if fewer than 7 (the greatest possible number) "extras" are present they are the most posterior nuclei in the embryo (except for the normal non-eliminator).

Table 10. *Centrifugation effects on individual embryos* (see text)

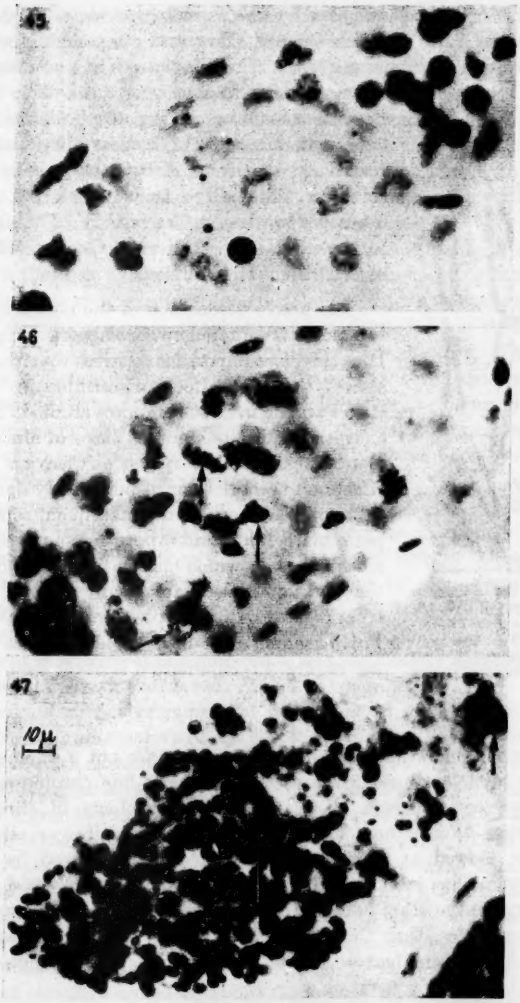
Amount of displacement		
Slight	Moderate	Large
1—2 (2; 6)	3 (1; 5)	5—6 (4; bl.)
2 (mat.; 5)	2—3 (4; bl.)	7 (2; 7)
2 (4; 5)	1—2 (4; 8)	6—7 (3; 8)
1 (3; 6)	2—3 (2; 6)	7 (2; 5)
		6—7 (3; 6)
		6—7 (3; 6)

Examples of the embryos showing more severe displacement are shown in Fig. 45—47. These are all orientated diagonally across the page, with nurse-cell (anterior) end in the lower left-hand corner. Almost the whole of each embryo is shown. In all three embryos the nuclei are displaced into less than half of the space they normally occupy. Fig. 45 and 46 show embryos 1 and 2 respectively in column 3, Table 10. While the stage of such embryos cannot be exactly given, this does not affect the significance of the result of centrifugation. In Fig. 45 (posterior displacement) 11 of about 20 interphase non-eliminators are in focus. Many have already begun to degenerate. Only the highly pycnotic polar body derivatives are in the anterior part of the embryo. Fig. 46 even more clearly shows the almost complete lack of chromosome loss in an embryo in which the nuclei are displaced anteriorally. The uppermost arrow indicates a normal, and the middle arrow a degenerating metaphase. There are a total of about 9 of the former and 4 of the latter (in addition to degenerating polar body derivatives).



Figs. 43 and 44. Centrifuged embryos; Feulgen whole mounts. See text

The fate of these embryos is clear. Chromosome elimination analogous to the normal sort never occurs after Cleavage IV. The extra non-eliminators always degenerate by the late blastula stage. As noted above, only normal embryos survive to beyond gastrulation. These facts make it clear that only embryos with a sufficient number of normal somatic nuclei can escape dissolution. This is verified by Fig. 47 — an embryo equivalent to a later stage of the one in Fig. 46. All the nuclei are pycnotic and complete dissolution is at hand. The arrow, upper left, indicates a part of another embryo. In the lower right corner are a few normal somatic blastula nuclei from still another embryo. One or two of these nuclei are not overlapped, and may be compared in size and density with the non-eliminators discussed above.



Figs. 45—47. Centrifuged embryos; Feulgen whole mounts. See text

It is interesting that some chromosome loss is occasionally found in the *anterior* nuclei in embryos like that shown in Fig. 46. For in that

figure, the metaphase indicated by the lowest arrow shows (with higher magnification) only 22 chromosomes. How and when such elimination may take place is shown by Fig. 48. This anaphase is in a position in the embryo similar to that occupied by the 22 chromosome metaphase in Fig. 46; both the magnitude and direction of nuclear displacement is essentially the same in both of these embryos. However, the embryo from which Fig. 48 was taken is doubtless in Cleavage IV. The similarity to the same stage in normal Cleavage III elimination (Fig. 21) is obvious.

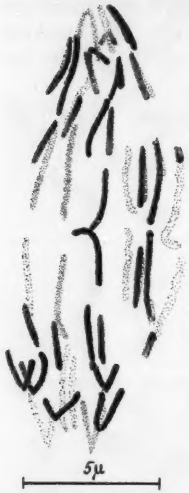


Fig. 48. From Feulgen whole mount of a centrifuged Cleavage *IV* embryo. Mid-anaphase in an anterior nucleus. Not all chromosomes are shown

The above description of the effects of centrifugation has centered on chromosome elimination. It is therefore important to stress that these were almost the only effects discernible in cleavage stages among over 600 embryos studied. The only exceptions were one or two cases of almost complete blocking in metaphase at Cleavage IV, and effects on the behavior of polar body derivatives that will be described in a subsequent report. It is particularly important to note the complete absence of embryos in which elimination was induced in the division of the primordial germ-cell. This is true regardless of its position at Cleavage IV. Furthermore, even if it divides at Cleavage IV (as in Fig. 43) elimination is never seen.

D. Discussion

Whether the somatic chromosomes are or are not a distinct group, without homologs among the rest of the germ-line chromosomes, has important consequences in relation to the problem of chromosome elimination. For if homologs do exist, then chromosome elimination must be viewed as a sort genome segregation followed by somatic reduction, as has been reported most recently by Gläss (1956, 1957) in rat liver. On the other hand, if these are specific, non-homologus groups of chromosomes, then their differential behavior can be viewed as an inherited, constant feature of the two groups of chromosomes that is repeated in every generation.

This question could not be settled by the previously available data on chromosome number and morphology given by Kahle, Reitberger, Kraczkiewicz and White. The data behind White's (1950) concept of a lack of homology are clearly insufficient to establish it. In the pre-

sent study a concentrated attack has for the first time been directed on this problem, in the hope of a final solution. The results make it certain that half of the germ-line chromosomes cannot be homologous with any of the somatic chromosomes. Nevertheless, the possibility that each of the four somatic chromosomes is at least tetraploid in the germ-line nuclei is not excluded by these data. The data show that there exists little homology of whole chromosomes between these two groups, but they do not prove that *none* exists. It would appear that only genetic data can give a final answer to this question.

The immediate cause of chromosome elimination has been ascribed by WHITE (1950) to one or another type of failure in splitting of the ends of the elimination chromosomes so that the daughter chromatids cannot separate despite a normal anaphase tension exerted upon them. This was said to apply to all the cecidomyids then investigated. This is an attractive hypothesis because of the appearance of the eliminated chromosomes from mid-anaphase to telophase. However, evidence from the most diverse sources argues against it. For instance, the present study shows that such a stage is a regular feature of mitosis here, even when a perfectly normal division follows.

Furthermore, REITBERGER in 1939 demonstrated that the daughter chromatids of the eliminated chromosomes separate completely in very early anaphase. I have shown that this is also true in *Miastor* and further demonstrated that this separation can be seen even in late metaphase. Again, experimental separation of daughter chromatids can be achieved by pressure; significantly, all the chromosomes of the complement at an elimination metaphase respond exactly alike, whatever the degree of separation achieved.

However, the fact remains that at mid-anaphase, the chromatids of eliminated chromosomes at least touch at the ends, and do not evince further poleward movement. Clearly, such behavior could result from some type of fusion of daughter chromatid ends, and/or from inadequate anaphasic forces toward the poles. The latter might be due to a failure in normal chromosomal fiber action. The results point to the second possibility alone as the immediate cause of chromosome elimination. First of all, some daughter chromatids at Cleavage IV remain separate throughout anaphase and nevertheless fail to move to opposite poles. Also, Cleavage III elimination in *Miastor* is instructive; it clearly shows a tremendous range of anaphase movement in individual chromatids that almost certainly separated at the same time. In this case the immediate cause seems to lie in a partial or complete failure in production of normal anaphase tension on the chromatids. The experiments reported in the present paper make it possible to generalize this behavior to account, also, for chromosome elimination like that in Cleavage IV.

As I have shown, Cleavage III elimination can, rarely, have exactly the appearance of elimination at Cleavage IV. Furthermore, experimentally induced increase of the variablity in elimination at Cleavage IV produces exactly the same result as the natural variation at Cleavage III. Thus the only simple interpretation is that the same mechanisms are operative at both cleavages. In Cleavage IV, however, there is complete and uniform action of this mechanism, whereas at Cleavage III it is not yet completely stabilized, and thus much variation is possible.

Therefore it seems probable that failure of anaphase tension alone accounts for chromosome elimination. An apparent exception may be cited to clinch this argument. Genuine daughter chromatid interlocking does occur in rare individual chromatid pairs at Cleavage III. However, if mid-anaphase tension is normal, such chromatids, regardless of where they lie on the spindle, are stretched nearly to opposite poles. The eliminated chromosomes at Cleavage IV anaphase are neither separated into their daughter chromatids nor are they stretched. This is a strong argument that the normal tension is not produced. Further, daughter chromatids at cleavage three almost always separate with no more stretching than is normally present (compare Fig. 21 and 16—17). All this evidence makes it certain that chromatid interlocking only very rarely plays any causative role in chromosome elimination in these midges, and also that the immediate cause must be sought in some failure of the forces that produce the normal mid-anaphase tension.

This conclusion can be extended to the rest of the cecidomyids in which something is known about the pattern of elimination (*Oligarces*, *Phytophaga* and *Monarthropalpus*). It is of interest that the second elimination in *Oligarces* (Reitberger 1939) apparently is very similar to Cleavage III elimination in *Miastor*.

Other examples of chromosome elimination occur in the fungus gnat *Sciara* (du Bois 1933), in the orthocladiine chironomids (Bauer and Beermann 1952; Beermann 1956) and in several plants, of which *Xanthisma* (Berger et al. 1955) is the most recent one in which the process of elimination has been studied. In all of these except the last, failure of daughter chromatid splitting has been held responsible for elimination. Only in *Sciara* is there evidence that this may be true, and in all of them reinvestigation is warranted before this can be accepted as proven. In *Xanthisma*, all that could be said was that elimination was due to "lagging of the supernumeraries at mitosis" (Berger et al. 1955).

Two examples of anaphase behavior very similar to that shown to be effective here in causing chromosome elimination are on record. These are the behavior of the X-chromosome in the bug *Protenor* (Schrader 1935) and of the X^1X^2Y chromosomes in the several mantid families in which such a sex chromosome complex occurs (Hughes-Schrader 1950;

1953). In both of these, at one particular division or cycle of divisions, the sex chromosomes separate in synchrony with the autosomes. However, during mid-anaphase they lag behind the autosomes and are tardily or never included in the telophase nuclei. They are not eliminated in either case, and thus they parallel in behavior many of the chromosomes at Cleavage III in *Miastor*, which show some, but not complete, inhibition of mid-anaphase movement. Further striking similarities are the presence of apparently normal chromosomal fibers and normal chromatid—pole orientation. HUGHES-SCHRADER (1950) believes that the mechanism behind this behavior is "... an inactivation of the chromosomal fibers of the sex-chromosomes during the last half of anaphase". This may very well be the cause of what I have termed "insufficient anaphase tension". In any event, the mechanism is almost certainly the same in these diverse groups, as RIS (1943) already suspected for the examples then known.

The fact that DNA synthesis is normal in chromosomes about to be eliminated is good to know, if not very helpful in suggesting what may *not* be normal. In view of the late mitotic stage at which failure occurs, it can hardly be surprising that DNA synthesis is in no way implicated.

WHITE (1950) has suggested on admittedly weak morphological grounds that the eliminated chromatin increases in mass after elimination. This, if true, would make possible an important addition to PAINTER's (1945) theory that eliminated material is functional in supplying nucleotides for the rapid cleavages that follow (and preceed!) elimination. If any extensive replication of this material occurs, one would expect some evidence of this by Cleavage IV, fully two chromosome synthesis cycles (for the somatic nuclei) after elimination. The data presented show that as far as DNA is concerned, no such synthesis occurs.

The centrifugation experiments constitute the first attempt at experimental analysis of the factors that control chromosome elimination. The effects of centrifugation on elimination at Cleavages III and IV considered together are very clear and specific. First there is the evidence that displacement of nuclei and cytoplasm in any manner or at any time never initiates elimination where it would not normally occur, i.e., in the most posterior—the primordial germ-cell—nucleus. On the contrary, the only effect of centrifugation on elimination is to produce extra non-eliminators. This effect is very specific. Thus the number of these "extras" is strictly proportional to the amount of displacement of the nuclei. Furthermore, the extras are always the most posterior nuclei in the embryo, except for the one equivalent to the primordial germ-cell nucleus.

These facts support the hypothesis that the posterior cytoplasm has properties that actively inhibit elimination that normally is certain to

occur at a certain general time in development. On this basis the limitation of extra non-eliminators to the posterior end when there is little displacement is easily understood. Under such circumstances relatively little mixing of the posterior and anterior cytoplasm has taken place, and thus the inhibitors influence only a few of the more posterior nuclei. It has been shown that the amount of nuclear displacement indicates the amount of cytoplasmic displacement, and hence the amount of mixing of anterior cytoplasm with that normally segregated in the primordial germ-cell area. Therefore the hypothesis will explain not only that more "extras" result from more rigorous nuclear displacement, but also the otherwise puzzling fact that displacement *either* anteriorally or posteriorally gives the same results. The latter is intelligible because centrifugation in either direction may be expected to produce mixing of anterior and posterior cytoplasm.

The gradient in chromosome elimination found normally at Cleavage III (KAHLE 1908 and the present study) could be interpreted as due to cytoplasmic factors *causing* elimination. However, it is just as easily interpreted in consonance with the above hypotheses as the result of chromosome elimination before the factors inhibiting elimination have been completely localized around the primordial germ-cell. This interpretation and indeed the hypothesis as a whole, are strengthened by the observation that this gradient can be recreated at Cleavage IV by centrifugation (Fig. 46 and 48).

These factors preventing chromosome elimination could just as well (and less negatively!) be called "factors permitting normal mitosis". The presence of cytoplasmic factors initiating chromosome elimination is not excluded by the present data. However, the fact that no sort of nucleocytoplasmic relocation gave elimination in the division of the primordial germ-cell is considerable, though negative, evidence against this idea, and also against any *double* gradient conception of chromosome elimination.

It was hoped that the qualitative cytochemical data presented might give some idea about the nature of the "inhibitory factors". However, the most striking result of these studies is the quite *even* distribution of RNA, protein and glycogen. Such relatively gross studies, however, hardly preclude the involvement of small differences in the distribution of these chemicals or in a particular protein, for instance, in the observed effects. Possibly, electron microscopical examination may disclose subtle qualitative or quantitative differences between the anterior and posterior cytoplasm. The results of the present study already make it doubtful that the polar granules can be concerned in any very direct way.

The centrifugation results illustrate a clear difference between the cecidomyids and the ascarids in the factors controlling loss of somatic

chromatin. In the latter (BOVERI 1910, HOGUE 1910) centrifugation studies have indicated the presence of cytoplasmic factors which *cause* chromatin loss (in this case, loss of *parts* of chromosomes). Indeed, the results of KING and BEAMS (1938) strongly indicate, as they suggest, that these are the *only* factors present (i.e., cytoplasmic *inhibitors* of loss are absent) and that the rate at which these factors are formed controls the time at which elimination occurs. It is not surprising that this difference between *Ascaris* and *Miastor* exists. Chromatin loss in the ascarids involves the breaking up of a compound chromosome into its components — some of which lack a kinetochore and are therefore lost. This fragmentation, *in itself*, has nothing to do with chromosome anaphase behavior, and the involvement of cytoplasmic factors is not amazing. However, in the cecidomyids, the loss is due to actual failure in anaphase — probably of the chromosomal fibers. In view of the considerable body of evidence that the formation and the behavior of these fibers is under chromosomal control (HUGHES-SCHRADER 1924, reviewed by SCHRADER 1953), it is not surprising that my evidence indicates no cytoplasmic initiation of elimination. It is surprising, however, that cytoplasmic factors can prevent this predisposition to failure.

The analysis of chromosome elimination has been advanced in two major areas. First, we now have descriptive data indicating precisely what is aberrant. Secondly, the first attempt at experimental analysis has provided facts that clearly suggest at least part of the mechanisms which limit elimination to only certain nuclei. The latter also indicates that the cecidomyids may provide much useful information on the relative roles played by nucleus and cytoplasm in the control of anaphase movement.

Note added in proof: I. GEYER-DUSZYŃSKA's interesting preliminary report [Bull. Acad. Sci. Poland **6**, 371—375 (1958)] of experimental studies on chromosome elimination in the cecidomyd *Wachtliella* has recently been called to my attention. She has also studied the developmental effects of centrifugation, and her interpretation differs somewhat from that presented here. It is hoped that her forthcoming paper presenting the data in full will clarify whatever real differences may exist.

Summary

1. The descriptive cytology of chromosome elimination has been reinvestigated. The results show that most of the eliminated chromosomes are definitely not homologous with the somatic chromosomes.

2. Evidence from both descriptive and experimental sources shows beyond doubt that the errant feature of the chromosomes eliminated is a failure in the production of normal mid-anaphase tension. Interlocking or sticking of daughter chromatid ends is only rarely if ever responsible for chromosome elimination in the *Cecidomyidae*.

3. The normal amount of DNA is synthesized just prior to chromosome elimination, and hence no alteration in the normal DNA relationships is involved in the chromosomal behavior here. The distribution of RNA, protein and polysaccharides has also been studied; nothing to indicate their direct involvement in chromosome elimination was detected.

4. Centrifugation studies provide support for the hypothesis that in the cecidomyids chromosome elimination is a largely autonomous act of the chromosomes — it can be prevented but not initiated by cytoplasmic factors. Nucleocytoplasmic interaction therefore will account for the limitation of elimination to only the future somatic nuclei, by permitting normal division of the primordial germ-cell chromosomes.

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TEMPERATURE EFFECT ON CHIASMA FREQUENCY IN THE BLUEBELL ENDYMION NONSCRIPTUS

By

J. YANNEY WILSON

With 2 Figures in the Text

(Eingegangen am 20. November 1958/4. Februar 1959)

Introduction

In a recent review of the literature on the effect of temperature on chiasma and cross-over frequency (WILSON 1957, 1959a) the present author pointed out that there exists a number of varied and apparently conflicting reports on the subject. This was shown to the largely due to (a) the different periods of time which have been used arbitarily in the different experiments and (b) the fact that cross-over or chiasma frequency is ordinarily rather variable among individuals of a species or a stock of experimental material. A remedy to this situation was proposed and its merits over previous experiments discussed. This method consisted in (I) giving the plants at each temperature just enough time for meiosis to be fully completed at that temperature, and (II) using clonal material whose homogeneity in chiasma frequency should be previously verified. It is clear from these conditions that before employing in the experiments just enough time that is taken by meiosis at any particular temperature, it is necessary first to know what this time is. Reference to the literature showed that it seems this has not been reported before (see WILSON 1959b). Thus the times taken by meiosis at the different temperatures which were used in the present experiments were first estimated (WILSON 1959b). Again in order to certify that in the experimental material to be used, chiasma frequency was uniform, a preliminary study was made by comparing the mean chiasma frequency in the individual members of each clone (WILSON 1957). This showed that the mean chiasma frequencies of members of each clone do not differ significantly from each other while they differed clearly from those of another clone.

After satisfying these pre-requisites for carrying out the experiments under the two conditions proposed above, the way was then clear to perform these experiments which are reported in this paper.

Material and Method

Bulbs of the Bluebell, *Endymion nonscriptus* (L.) GARCKE came from Madingley Wood, Cambridge, during the winter of 1955/6, when meiosis was proceeding in this species. The clones were selected and marked in the field during the spring of 1955.

The method of handling the material in these experiments was the same as that which was used in the experiments to estimate the duration of meiosis at different temperatures (WILSON 1959b). The essential feature of this was the use of petri-dishes instead of pots, and absorbent cotton-wool in place of soil.

The experimental plan consisted of placing individual plants of a given clone at different temperatures, and repeating this with other clones of the same number of plants. By this means it was expected that the pattern of the effect produced by the different temperatures would be demonstrated irrespective of the original mean chiasma frequency of any particular clone.

Experimental Data and Analysis

A. Experiments with clones of 6 individuals

I. Clone A_{18}

The full data of frequency distribution of chiasmata are given in Table 1 and the mean nuclear chiasma frequencies at the different temperatures used are given below and presented graphically in Fig. 1.

	Temperature °C					
	0°	5°	10°	15°	20°	25°
Mean chias. freq.	17.10	16.03	15.87	15.43	15.50	14.13

Table 1. *Chiasma frequency distribution data for experiments with clones of 6 individuals.* (Clones A_{18}, N_{11} and N_{30} [19. 12. 55])

Clone and temper.	No. of PMCs with total chiasmata of												Total Xta	No. of cells	Mean ± SE
	11	12	13	14	15	16	17	18	19	20	21	22			
A_{18} 0°	—	—	—	1	3	8	6	6	4	2	—	—	513	30	17.10 ± 0.281
5°	—	—	3	2	6	10	2	3	4	—	—	—	481	30	16.03 ± 0.323
10°	—	1	2	3	5	10	4	3	1	1	—	—	476	30	15.87 ± 0.324
15°	—	1	—	7	10	7	1	2	2	—	—	—	463	30	15.43 ± 0.286
20°	—	—	2	4	10	7	5	2	—	—	—	—	465	30	15.50 ± 0.239
25°	2	6	3	9	4	2	1	1	2	—	—	—	424	30	14.13 ± 0.389
N_{11} 0°	—	—	—	—	4	10	9	4	2	1	—	—	503	30	16.77 ± 0.228
5°	—	—	1	1	1	7	12	3	5	—	—	—	507	30	16.90 ± 0.260
10°	—	—	2	3	5	10	4	5	1	—	—	—	480	30	16.00 ± 0.270
15°	—	—	1	6	7	7	2	1	—	—	—	—	366	24	15.25 ± 0.243
20°	—	—	2	6	5	8	4	4	1	—	—	—	472	30	15.73 ± 0.291
25°	—	1	2	4	5	4	3	1	—	—	—	—	310	20	15.10 ± 0.347
N_{30} 0°	—	—	—	—	1	3	4	6	5	7	1	3	561	30	18.70 ± 0.339
5°	—	—	4	5	9	3	4	2	2	1	—	—	467	30	15.57 ± 0.348
10°	—	—	5	8	4	9	3	—	1	—	—	—	451	30	15.03 ± 0.273
15°	1	2	4	12	4	4	2	1	—	—	—	—	431	30	14.37 ± 0.286
20°	2	2	3	8	10	3	2	—	—	—	—	—	429	30	14.30 ± 0.276
25°	1	4	10	7	5	2	1	—	—	—	—	—	411	30	13.70 ± 0.250

Comparison of these mean values by the analysis of variance is given below.

Item	Degrees of freedom	Sum of squares	Mean square	*F*	*P*
Between temperatures	5	139.83	27.966	7.728	<0.001
Within temperatures .	174	557.48	3.204		
Total	179	697.31			

From the values of $F=7.728$, and $P<0.001$ it is clear that the mean chiasma frequencies at the different temperatures are significantly different.

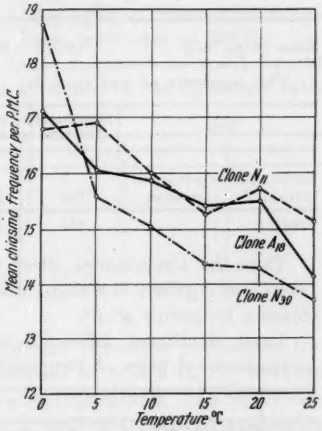

Fig. 1. Graphical representation of the effect of temperature on mean nuclear chiasma frequencies in three clones A_{18}, N_{11} and N_{30} of *Endymion nonscriptus* (L.) GARCKE each with six individuals

Since significant differences have been demonstrated, comparisons were then made between pairs of the temperature means. Until recently the technique used has been by the STUDENT'S *t*, introduced by Sir R. A. FISHER. But this has now been generally abandoned in favour of the studentised range in samples of *n* individuals (IHM 1955, 1957). This involves calculation of new least significant differences (L. S. D.) by multiplying the standard deviations of the *n* means to be compared by the 0.05 p.c. value of the studentised range in samples of *n*. For this purpose the differences between the means in the present data were arranged in a difference table, as shown below, and an asterisk is used to denote the significant differences.

	0°	5°	10°	15°	20°	25°
0°	—					
5°	1.07	—				
10°	1.23	0.16	—			
15°	1.67*	0.60	0.44	—		
20°	1.60*	0.53	0.37	−0.07	—	
25°	2.97*	1.90*	1.74*	1.30	1.37*	—

* L. S. D. =1.32.

This analysis shows that the first significant drop in chiasma frequency occurs at 15° C after which there is a slight non-significant

rise at 20° followed by a significant fall at 25° C. The only significant fall involving a 5° difference in temperature is 20—25° C.

II. Clone N_{11}

The full data are in Table 1 and the mean values which are presented graphically in Fig. 1 are given below.

	Temperature °C					
	0°	5°	10°	15°	20°	25°
Mean chias. freq.	16.767	16.900	16.00	15.250	15.830	15.100

The analysis of variance is:

Item	Degrees of freedom	Sum of squares	Mean square	F	P
Between Temperatures	5	73.54	14.707	7.168	<0.001
Within Temperatures	158	324.24	2.052		
Total	163	394.78			

Thus the temperature effect is significant, $F=7.168$ ($P<0.001$). Like Clone A_{18} there is a slight rise at 20° C, but unlike it is the rise in chiasma frequency at 5°.

Least significant difference was calculated as for Clone A_{18} and comparisons of pairs of temperatures made as shown below:

	0°	5°	10°	15°	20°	25°
0°	—					
5°	−0.133	—				
10°	0.767	0.900	—			
15°	1.517*	1.650*	0.750	—		
20°	0.937	1.070	0.170	−0.580	—	
25°	1.667*	1.800*	0.900	0.150	0.730	—

* L. S. D. = 1.111.

This analysis shows that like Clone A_{18} the first significant drop in chiasma frequency was at 15° C. No temperature difference of 5° showed a significant decrease in temperature, the minimum range here being 10° i.e. between 5 and 15° C.

III. Clone N_{30}

The full data are in Table 1 and the mean chiasma frequencies, shown graphically in Fig. 1, are given below:

	Temperature °C					
	0°	5°	10°	15°	20°	25°
Mean chias. freq.	18.000	15.667	15.033	14.367	14.300	13.700

The analysis of variance is:

Item	Degrees of freedom	Sum of squares	Mean square	F	P
Between Temperatures	5	483.90	96.780	36.438	<0.001
Within Temperatures	174	462.21	2.656		
Total	179	946.11			

Here again the temperature effect is highly significant: $F = 36.438$ ($P < 0.001$). Like Clone A_{18} there is a sharp fall in chiasma frequency between 0 and 5° C, but unlike N_{11} there is no rise at 20° C.

Differences between the means were compared as usual by the calculated least significant difference as shown in the difference table below:

	0°	5°	10°	15°	20°	25°
0°	—					
5°	2.333*	—				
10°	2.967*	0.634	—			
15°	3.633*	1.300	0.666	—		
20°	3.700*	1.367*	0.733	0.067	—	
25°	4.300*	1.967*	1.333*	0.667	0.600	—

* L. S. D. = 1.199.

The analysis shows the first significant reduction in chiasma frequency to be between 0 and 5° C, followed by 5 and 15° C. Between 15° and 25° C the reduction is no more significant. The significant drop in chiasma frequency between 0 and 5° C has an interesting relevance to the observations of ELLIOTT (1953) and will be commented on later in the discussion.

Joint analysis of clones A_{18}, N_{11} and N_{30}

The mean chiasma frequencies at the different temperatures for the three clones were analysed as in a 3×6 randomised block experiment in order to find out what effect the clone differences have had in relation to the temperature effect. The joint analysis of variance is:

Item	Degrees of freedom	Sum of squares	Mean square	F	P
Between temperatures	5	18.589	3.7178	7.362	<0.001
Between clones . .	2	1.400	0.7000	1.386	
Error	10	5.050	0.5050		
Total	17	25.039			

The analysis shows the overall temperature effect in the three clones to be significant $F = 7.363$ ($P < 0.001$). The differences between the clones, on the other hand, are not significant $F = 1.386$ (5% pt. $= 4.10$), but it should be pointed out that if even this were significant

when compared with the error variance, this would not detract from the result of the temperature effect in view of the experimental set-up itself; so that no further test of significance would be necessary, such as that of comparing the temperature variance with the between clone variance.

Pairs of overall temperature mean chiasma frequencies were compared in the usual way by means of the least significant difference and the difference table:

	0°	5°	10°	15°	20°	25°
0°	—					
5°	1.35	—				
10°	1.89*	0.54	—			
15°	2.34*	0.99	0.45	—		
20°	2.31*	0.96	0.42	+0.03	—	
25°	3.21*	1.86*	1.32	0.87	0.90	—

* L. S. D. = 1.428

The difference table shows that there is a remarkable drop in chiasma frequency between 0 and 5° C although this does not appear to be significant. At 10° C the fall in chiasma frequency becomes significant. The other significant decrease in temperature is between 5 and 25° C although between 10 and 25° C the fall is remarkable but not significant.

B. Experiments with clones of 4 individuals (19. 12. 55)

Only two clones of four individuals were used at temperatures of 5, 10, 15 and 20° C. But unfortunately, all the plants at 15° C died

Table 2. *Chiasma frequency distribution data for experiments with clones of 4 individuals* (Clones A_5 and A_7 [19.12. 55])

Clone and temperature	No. of PMCs with total chiasmata of										Total Xta	No. of cells	Mean ± SE
	11	12	13	14	15	16	17	18	19	20			
A_5 5°	—	—	—	—	1	7	2	2	3	—	254	15	16.93 ± 0.344
10°	—	1	—	3	10	8	3	2	—	—	419	27	15.52 ± 0.247
20°	—	1	6	8	4	4	3	1	—	—	395	27	14.63 ± 0.300
A_7 5°	—	—	1	4	5	6	5	3	1	2	438	27	16.22 ± 0.351
10°	—	—	3	3	6	3	3	4	1	—	361	23	15.70 ± 0.374
20°	—	1	2	8	6	3	5	1	2	2	469	30	15.63 ± 0.382

for unknown reason, so the experiment was treated like one with only three plants per clone. The full data are given in Table 2 and summarised below for both clones:

Clone	5°	10°	20°
A_5	16.93	15.52	14.63
A_7	16.22	15.70	15.63

Clone A_3

The analysis of variance for this clone is given below:

Source of variation	Degrees of freedom	Sum of squares	Mean square	*F*	*P*
Between temperatures	2	50.42	25.21	12.732	<0.001
Within temperatures .	66	130.80	1.98		
Total	68	181.22			

Thus the between temperature differences are statistically significant and there is a progressive fall in mean chiasma frequency.

Pairs of temperature means were compared in the usual way as shown below:

	5°	10°	20°
5°	—		
10°	1.41*	—	
20°	2.30*	0.89	

* L.S.D. = 0.998

This shows that between 5° and 10° C the difference is significant, while that between 10° and 20° C is not quite so. (Difference = 0.98, L.S.D. = 0.998.)

Clone A_7

The analysis of variance is as follows:

Item	Degrees of freedom	Sum of squares	Mean square	*F*	*P*
Between plants . . .	2	5.69	2.845	0.7701	0.2
Within plants	77	284.51	3.694		
Total	79	290.20			

Here there is no significant effect of temperature although the mean chiasma frequencies are lower at 10 and 20° C than at 5° C.

C. Experiments with clones of 3 individuals (23. 12. 55)

In this experiment four clones, each of three plants were treated at 5, 15 and 25° C. Some of the plants were found to have died and could therefore not be scored. The full data are given in Table 3 and the mean chiasma frequencies are given below:

Clone	5°	15°	25°
A_8	16.07	15.07	10.70
A_{13}	18.20	15.81	—
A_{19}	15.47	16.20	—
N_9	15.10	—	12.90

Table 3. *Chiasma frequency distribution data for experiments with clones of 3 individuals* (Clones A_9, A_{13}, A_{19} and N_9 [23. 12. 55])

Clone and temperature	No. of PMCs with total chiasma of														Total Xta	No. of cells	Mean ± SE
	8	9	10	11	12	13	14	15	16	17	18	19	20	21			
A_9 5°	—	—	—	—	—	—	5	7	6	8	2	1	1	—	482	30	16.07 ± 0.279
15°	—	—	—	—	1	3	10	8	2	2	1	3	—	—	452	30	15.07 ± 0.335
25°	3	—	1	2	2	1	1	—	—	—	—	—	—	—	107	10	10.70 ± 0.684
A_{13} 5°	—	—	—	—	—	—	—	—	1	2	2	4	1	1	182	10	18.20 ± 0.389
15°	—	—	—	—	—	1	3	9	5	6	2	1	—	—	427	27	15.81 ± 0.272
A_{19} 5°	—	—	—	—	—	3	3	9	8	6	1	—	—	—	464	30	15.47 ± 0.238
15°	—	—	—	—	—	3	2	4	7	6	7	1	—	—	486	30	16.20 ± 0.305
N_9 5°	—	—	—	—	—	2	7	11	7	2	1	—	—	—	453	30	15.10 ± 0.282
25°	—	1	2	3	3	6	3	3	1	—	—	—	—	—	258	20	12.90 ± 0.397

Of these clones, A_{19} seems to have behaved curiously in showing a higher value at 15° C than at 5° C, otherwise, allowing for the missing data, the other clones show a progressive fall in chiasma frequency with increasing temperature.

The analysis of variance and *t*-tests carried out for each clone are shown below:

Clone A_9

Source of ariation	Degrees of freedom	Sum of squares	Mean square	*F*	*P*
Between temperatures	2	218.01	109.005	35.14	< 0.001
Within temperatures .	67	207.84	3.102		
Total	69	425.85			

The analysis shows significant differences between temperatures. Pairs of temperature means were compared in the usual way as shown below:

	5°	15°	25°
5°	—		
15°	1.00	—	
25°	5.37*	4.37	—

* L. S. D. = 1.241

This shows that while the decrease in temperature between 5 and 15° is not significant, that between 15 and 25° C is remarkably so.

Clone A_{13}

Here the *t*-test shows that the difference (2.39) between the mean chiasma frequencies at 5 and 15° C is significant ($t = 4.714$, $P < 0.001$).

Clone A_{19}

Here the difference (0.733) between 5 and 15° C is not quite significant ($t = 1.894$, 5% $pt = 2.000$).

Clone N_9

Here the difference between 5 and 25° C (2.20) is highly significant ($t = 5.327$, $P < 0.001$).

D. Experiments with clones of two individuals

The full data of results for all experiments under this category are given in Table 4.

I. Experiment 0—10° C

Three clones were used at these two temperatures. The mean chiasma frequencies and results of their comparisons are given below:

Clone	0°	10°	Diff.	t	P
A_{22}	18.82	16.93	1.89	3.36	0.01—0.001
B_{12}	16.60	16.67	0.07	0.17	0.9—0.8
B_{21}	17.30	16.71	0.59	1.42	0.1—0.05

This set of experiments has shown how the response of different clones differ under the same temperatures. Clone B_{12} shows essentially no change in chiasma frequency at 0 and 10° C whilst clone A_{22} shows a remarkably great decrease at 10° compared with the value at 0° C.

II. Experiment 10—25° C

Three clones were also used in this set of experiments. The mean chiasma frequencies and results of their comparisons are given below:

Clone	10°	25°	Diff.	t	P
A_{20}	16.16	15.83	0.33	0.816	0.5—0.4
C_5	17.00	9.87	7.13	12.82	<0.001
C_6	15.73	12.97	2.96	6.04	<0.001

This set of experiments has indicated clearly a decrease in chiasma frequency with rising temperature; once again there have been differences in degree of response, in clone A_{20} the reduction is non-significant whereas it is highly so in the other two clones. It may be remarked that the decrease in chiasma frequency in clone C_5 (7.13) between 10° and 25° C is the greatest recorded in the present experiments.

III. Experiment 15—30° C

This set was carried out with two clones, and the mean chiasma frequencies and their comparisons at the respective temperatures are given below:

Clone	15°	30°	Diff.	t	P
A_{15}	16.47	13.67	2.80	5.39	<0.001
B_2	17.57	14.60	2.97	5.67	<0.001

Table 4. *Chiasma frequency distribution data for experiments with clones of 2 individuals*

	Clone and temperature	No. of PMCs with total chiasmata of																Total Xta	No. of cells	Mean ± SE
		6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22			
(I) Experiment 0—10° C	A_{22} 0°	—	—	—	—	—	—	—	—	2	—	1	1	9	3	6	5	1	527	28	18.82 ± 0.469
	10°	—	—	—	—	—	—	1	—	1	3	7	7	4	6	1	—	—	508	30	16.93 ± 0.321
	B_{12} 0°	—	—	—	—	—	—	—	—	2	6	8	4	6	4	—	—	—	498	30	16.60 ± 0.278
	10°	—	—	—	—	—	—	—	1	—	6	9	6	4	1	3	—	—	500	30	16.67 ± 0.305
	B_{21} 0°	—	—	—	—	—	—	—	—	—	1	7	3	5	2	2	—	—	346	20	17.30 ± 0.325
	10°	—	—	—	—	—	—	—	—	1	6	4	9	5	3	—	—	—	468	28	16.71 ± 0.262
(II) Experiment 10—25° C	A_{20} 10°	—	—	—	—	—	—	—	1	2	7	8	6	5	1	—	—	—	485	30	16.16 ± 0.259
	25°	—	—	—	—	—	—	—	5	3	2	6	10	4	—	—	—	—	475	30	15.83 ± 0.311
	C_5 10°	—	—	—	—	—	—	—	1	—	5	5	6	9	2	2	—	—	510	30	17.00 ± 0.296
	25°	3	4	3	4	4	3	4	2	2	1	—	—	—	—	—	—	—	296	30	9.87 ± 0.483
	C_6 10°	—	—	—	—	—	—	—	2	2	8	11	4	3	—	—	—	—	472	30	15.73 ± 0.235
	25°	—	—	—	1	2	6	4	6	3	5	1	1	1	—	—	—	—	389	36	12.97 ± 0.392
(III) Experiment 15—30° C	A_{15} 15°	—	—	—	—	—	—	—	1	4	3	5	10	4	2	1	—	—	494	30	16.47 ± 0.306
	30°	—	—	—	—	4	1	3	5	10	1	2	1	3	—	—	—	—	410	30	13.67 ± 0.419
	B_2 15°	—	—	—	—	—	—	—	—	—	3	4	6	10	4	3	—	—	527	30	17.57 ± 0.287
	30°	—	—	—	—	2	1	4	4	3	4	4	4	3	1	—	—	—	438	30	14.60 ± 0.454
(IV) Experiment 25—30° C	B_{23} 25°	—	—	—	—	—	—	—	3	3	3	1	3	2	1	—	—	—	248	16	15.50 ± 0.423
	30°	—	—	—	—	—	1	1	3	8	7	3	4	3	—	—	—	—	449	30	14.97 ± 0.323
	N_3 25°	—	—	—	—	—	—	—	2	2	4	4	4	2	2	—	—	—	320	20	16.00 ± 0.397
	30°	—	—	—	—	—	—	1	1	10	9	5	3	1	—	—	—	—	449	30	14.97 ± 0.237
	K_9 25°	—	—	—	—	—	—	—	2	2	3	6	5	3	—	—	—	—	334	21	15.90 ± 0.330
	30°	—	—	—	—	—	—	—	1	6	10	11	2	—	—	—	—	—	457	30	15.23 ± 0.162

While showing the obvious decrease in mean chiasma frequencies, the amount of chiasmata lost appear similar in these two clones, so that the relative means are maintained at 30° C (cp. above experiments).

IV. Experiment 25° – 30° C

All the experiments described so far show a decrease in mean chiasma frequency with temperatures increasing from 0 to 25° C. To complete the observations up to 30° C, three clones were used in this set of experiments to find the relationship at 25 and 30° C. The mean chiasma frequencies and the comparisons at the two temperatures are given below:

Clone	25°	30°	Diff.	*t*	*P*
B_{23}	15.50	14.97	0.53	0.931	0.4—0.3
N_3	16.00	14.97	1.03	2.37	0.05—0.02
K_9	15.91	15.23	0.63	1.957	ca. 0.05

Conclusion

The results indicate that beyond 25° C there is a still further decrease in chiasma frequency, although the amount of decrease was clearly significant in only one of the three clones. Thus it looks as if there is a progressive decrease in chiasma frequency in the bluebell with temperatures increasing from 0 to 30° when just adequate time is given for the full cycle of meiosis.

E. Effect of high temperature on internuclear variation in chiasma frequency

Quite apart from the above observations of effect of increasing temperatures on the mean chiasma frequency, another effect worth mentioning is on the variance of the internuclear chiasma frequency.

The data from the 1954/55 plants (WILSON 1957) seem to show that under natural conditions the variance about the nuclear mean chiasma frequency does not vary appreciably among individual plants or clones, although they seem to be much more similar for individuals within a clone. Again there was no correlation between the mean chiasma frequency of a plant or clone and its variance as shown by the regression analysis of the overall chiasma frequency and the pooled variance per „clone" (1954/55 data) given below:

Item	Degrees of freedom	Sum of squares	Mean square	*F*	*P*
Regression .	1	0.039	0.039	0.159	Not. sig.
Residual . .	23	5.627	0.245		
Total . . .	24	5.666			

This apparent absence of correlation between the mean chiasma frequency and its variance in the genotype has also been reported recently in different inbred lines of rye (REES and THOMPSON 1956).

The observation from the above temperature experiments with respect to internuclear variance in chiasma frequency was its significant increase which often accompanied a highly significant decrease in the mean chiasma frequency. Few examples are given here to illustrate this, the comparison having been carried out by means of the *F*-test for variance (each value of *F*, here has 29 and 29 degress of freedom):

Clone	Temp. °C	Variance	*F*	*P*
C_5	25	6.671	2.546	0.01—0.001
	10	2.621		
C_6	25	4.654	2.819	0.01—0.001
	10	1.651		
A_{15}	30	5.265	1.874	0.05—0.01
	15	2.809		
B_2	30	6.179	3.019	<0.001
	15	2.047		

Reference to the relevant frequency distribution data (Table 4) shows that the increase in inter-nuclear variance at higher temperatures arises by depletion in the frequency of cells originally with higher chiasma frequencies and the formation of cells with lower chiasma frequencies not represented at the lower temperature. The same mode of increase in internuclear variance has been reported recently as accompanying a decrease in mean chiasma frequency following inbreeding of rye (REES and THOMPSON 1956).

F. Comparison of chiasma frequency under natural conditions with the values at constant temperatures

Three clones of four plants each were used for this purpose, one individual of each being fixed immediately in the wood after the collec-

Table 5. *Chiasma frequency distribution for experiments with control under natural conditions* (29. 12. 56)

Clone and temperature	No. of PMCs with total chiasmata of													Total Xta	No. of cells	Mean ± SE
	9	10	11	12	13	14	15	16	17	18	19	20	21			
B_4 Control	—	—	—	—	—	1	—	4	3	8	10	3	2	544	30	18.13 ± 0.274
0°	—	—	—	—	1	3	5	9	8	3	1	—	—	483	30	16.10 ± 0.250
10°	—	—	—	1	—	2	2	7	6	6	1	—	—	411	25	16.44 ± 0.317
B_5 Control	—	—	—	—	2	7	9	6	3	1	2	—	—	462	30	15.40 ± 0.282
0°	—	—	—	—	1	5	5	8	5	4	2	—	—	481	30	16.03 ± 0.290
10°	—	—	—	—	2	6	7	5	2	1	—	—	—	349	23	15.09 ± 0.266
B_6 Control	—	—	—	2	3	8	8	4	2	2	1	—	—	448	30	14.93 ± 0.310
0°	—	—	1	—	3	6	8	5	5	2	—	—	—	455	30	15.17 ± 0.296
10°	—	—	—	1	4	2	2	3	1	1	—	—	—	205	14	14.64 ± 0.476
25°	—	1	2	2	3	3	2	1	1	—	—	—	—	186	14	13.29 ± 0.547

tion of the bulbs. Results could not be obtained for two of the bulbs at 25° C. The full data are given in Table 5 and the mean chiasma frequencies per cell are shown below:

Clone	Field control	0°	10°	25°
B_4	18.13	16.10	16.44	—
B_5	15.63	16.03	15.09	—
B_6	15.02	16.17	14.63	13.26

As far as the constant temperature experiments are concerned, it is clear that the same decrease in chiasma frequency with increasing temperature already demonstrated is shown here too. The main interest here then centres around the relationship between the control and the value at 0° C. In this respect the data the only case (B_4) where the difference between the two is significant shows the higher value in the control, as shown below:

Clone	0°-Field control	*t*	*P*
B_4	2.10	5.66	0.001
B_5	0.40	0.99	0.4—0.3
B_6	0.24	0.48	0.7—0.6

It is recalled that under natural conditions the temperature during the meiosis period fluctuates between + 0.5 and + 8° C; this would mean that the full cycle of meiosis (from leptotene to tetrad) takes a shorter time than at 0° C (WILSON 1958b). Thus on the basis of the results from the constant temperature experiments a lower value might be expected under natural conditions. The higher value rather found here might mean that chiasma formation is more effective under a fluctuating temperature than at a fixed one, even if the fluctuating temperature is much higher. This possible explanation was justified by the next experiment which follows.

G. Comparison of effect of constant and fluctuating temperatures

Two clones each of three plants were chosen for this experiment. One of each clone was placed at 15° C, another at 20° C and the third

Table 6. *Chiasma frequency distribution for experiment on effect of constant and fluctuating temperatures*

Clone and temperature	No. of PMCs with total chiasmata of													Total Xta	No. of cells	Mean ± SE
	9	10	11	12	13	14	15	16	17	18	19	20	21			
A_8 Room	—	—	—	—	2	2	5	8	9	2	1	1	—	485	30	16.17 ± 28.8
15°	—	—	—	—	1	2	8	5	5	5	—	—	—	472	30	15.73 ± 0.271
20°	—	—	—	3	6	4	4	6	5	2	—	—	—	447	30	14.90 ± 0.337
B_{17} Room	—	—	—	—	—	2	2	16	11	7	7	1	1	520	30	17.33 ± 0.289
15°	—	—	—	—	—	1	4	6	9	7	3	—	—	506	30	16.87 ± 0.238
20°	—	—	—	—	1	1	4	4	4	4	1	—	—	237	15	15.80 ± 0.341

kept in the laboratory where, as mentioned earlier, the room temperature during the period fluctuated daily between 14° and 21° C. Full data of the results for both clones are given in Table 6 and the mean chiasma frequencies shown below:

Clone	Room	15°	20°
A_8	16.17	15.73	14.90
B_{17}	17.33	16.87	15.80

Clone A_8

The analysis of variance for the means at the different temperatures is given below, and shows no overall significant differences ($F=1.72$, $P=0.2$).

Item	Degrees of freedom	Sum of squares	Mean square	F	P
Between temperatures	2	9.26	4.63	1.72	0.2
Within temperatures .	87	324.74	2.70		
Total	89	244.50			

Clone B_{17}

The analysis of variance is given below, and here there is an overall significant difference in mean chiasma frequency ($F=5.784$, $P=0.01$).

Item	Degrees of freedom	Sum of squares	Mean square	F	P
Between temperatures	2	23.54	11.77	5.784	0.01
Within temperatures .	72	146.54	2.035		
Total	74	170.08			

Pairs of mean differences were compared in the usual way as shown below:

	Room	15°	20°
Room	—		
15°	0.46	—	
20°	1.53*	1.07*	—

* L.S.D. = 0.964

Thus it seems that although the room temperature could reach 21° C by virtue of its fluctuation, it does not cause as much lowering of chiasma frequency as a constant temperature of 20° C.

Conclusions

1. Perhaps the principal conclusion that has emerged from the present temperature experiments is that between 0° and 30° C when clonal material of the bluebell, *Endymion nonscriptus* was given enough

time to allow meiosis to be completed under each of a range of temperatures, there was a gradual but consistent decrease in mean chiasma frequency with increasing temperature; the first significant decrease seems to occur between 0 and 5° C. Because of the precautionary method used in these experiments, the present results have repercussions on previous works in this field of study, and these will be considered later below.

2. Another observation is that a decrease in mean chiasma frequency appears to go with an increase in the inter-nuclear variance.

3. It is also demonstrated that different clones seem to differ in their degree of sensitivity to the same temperature. This seems to parallel the observations reported for different varieties of apples (HEILBORN 1930), strains of *Impatiens balsamia* (NAKAMURA 1936), different plants of *Vicia faba* (STRAUB 1938) and varieties of *Lilium longiflorum* (ENSWELLER and BRIERLEY 1943).

4. Finally constant temperature seems to be more deleteriosus to chiasma formation than fluctuating temperature.

Discussion

The present work is of special significance because it takes into account two rather important experimental precautions: (a) Clonal material whose homogeneity in mean chiasma frequency had already been verified and justified, has been used, (b) the time given to the plants at each temperature has been purposely chosen to correspond to the time normally required by a full cycle of meiosis. As proposed in the review of previous literature (WILSON 1959a), this approach was suggested as a way by which the experimental method in such investigations could be standardized so that if it was adopted by future workers using different materials, then legitimate comparison of results could be made.

It may be interesting to consider the apparently conflicting reports of previous workers in the light of the present results (in view of the two criteria employed here). Most of the previous works do not give the number of individuals used nor their mean chiasma frequencies even when the number of individuals are mentioned. As such the part played by the heterogeneity of chiasma frequency among the individuals cannot be effectively assessed. When we turn to the other factor, the time taken by meiosis (as estimated and used here) in relation to the various times that have been used by previous workers, the situation is a little better. In this respect, it may be mentioned that when the present estimated periods are compared with the few corresponding ones in the rather scanty literature on this (WILSON 1959b) there is the impression of general agreement in the times taken by meiosis in different

flowering plants. If this is the case, the present results may be taken to indicate that when just enough time is given for meiosis at different temperatures higher values of chiasma frequency are found at lower temperatures and lower ones at higher temperatures. But, as pointed out in the review of the literature on the subject, chiasma frequency was not constant at each temperature but changed with time. From the literature it appears that when an organism is placed at any new constant temperature, chiasma frequency remained high for the first few hours, then started to fall for some time and then began to rise again so that when full meiosis was through at that temperature, the "original" value may be exceeded in the case of low temperatures but not yet reached at higher temperatures; with more time than that required by meiosis the value at higher temperatures may continue to rise (probably as a result of adaptation) provided that high temperature was not deleterious for the organism, in which case the chiasma frequency would rather fall until the death of the organism. According to this premise, the results of the present experiments where just enough time was given for meiosis to be completed may be interpreted as shown in Fig. 2 below:

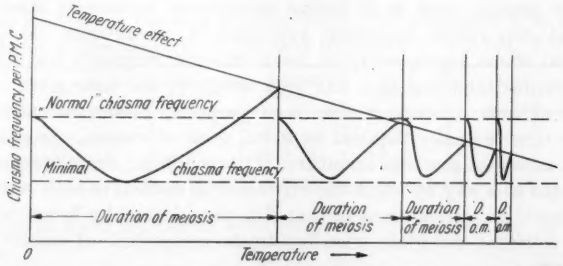

Fig. 2. Tentative schematic representation illustrating how the effect of temperature on mean nuclear chiasma frequency (when the material is given just enough time for the duration of meiosis at each temperature) is arrived at (see text)

This premise seems to help to explain some of the apparently conflicting reports in the literature (see WILSON 1959a). Thus those who reported a decrease in chiasma frequency with increasing temperature seem to have used relatively shorter periods (not more than twice the time for meiosis at the particular temperatures). Examples are 10° C for 5 days, 30 and 35° for 2 days (HEILBORN 1930), 34° for 1 day (STRAUB 1938) and 34° for 1 or 2 days (BARBER 1942).

Again those who reported the reverse of the above, that chiasma frequency increased with increasing temperature appear to have used rather longer periods. Examples are 20° for 2 or 3 weeks (MATSUURA

and HAGA 1942), 40° for 2 weeks (SWANSON 1942), 5° for 158—165 days, 20° for 43—47 days (ELLIOTT 1953).

In the case of data on U-shaped curves (WILSON 1959a), those of WHITE (1934) seem difficult to explain on the basis of the time given. But those of POLIAKOVA (1940) appear explicable mainly at the higher temperatures. He gave the plants 2 hours at each temperature. According to the present premise the higher the temperature the more quickly does the effect of the 2 hours' treatment fade away on return to room temperature (due to the faster rate of meiosis at higher temperatures) and hence a quicker recovery with consequent rise in chiasma frequency after 22 hours.

Future works on the lines used in present experiments would throw still more light on the results of previous workers.

Summary

1. Experiments have been conducted with the bluebell, *Endymion nonscriptus* (L.) GARKE to determine the effect of temperature on chiasma frequency using a proposed standardizing procedure by which (a) the material used is known to be homogeneous for chiasma frequency, and (b) just enough time is used at each temperature as is required for meiosis to be completed. These experiments showed a consistent but gradual decrease in mean chiasma frequency with increasing temperature.

2. A decrease in mean chiasma frequency is shown to be associated with an increase in inter-nuclear variance (see also REES and THOMPSON 1956).

3. Constant temperature seems to be more deleterious to chiasma formation than fluctuating temperature.

4. In the light of the present experimental results it has been possible to evaluate some of the apparently conflicting results of previous workers.

5. It is expected that future experiments along the lines of the present one would allow legitimate comparison of results from different materials.

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